(4)

CHEMICAL
RESEARCH,
DEVELOPMENT &
ENGINEERING
CENTER

CRDEC-CR-007

ACUTE ENVIRONMENTAL TOXICITY AND PERSISTENCE OF A CHEMICAL AGENT SIMULANT: 2-CHLOROETHYL ETHYL SULFIDE (CEES)

Dominic A. Cataldo, Ph.D.
Michael W. Ligotke, M.S.
Bruce D. McVeety, Ph.D.
Harvey Bolton, Jr., Ph.D.
Robert J. Fellows, Ph.D.
Shu-mei W. Li, M.S.
Peter Van Voris, Ph.D.
Eric A. Crecelius, Ph.D.
Jack T. Hardy, Ph.D.
PACIFIC NORTHWEST LABORATORY
Richland, WA 99352

Randall S. Wentsel, Ph.D.
RESEARCH DIRECTORATE

November 1988

U.S. ARMY
ARMAMENT
MUNITIONS
CHEMICAL COMMAND

Aberdeen Proving Ground, Maryland 21010-5423

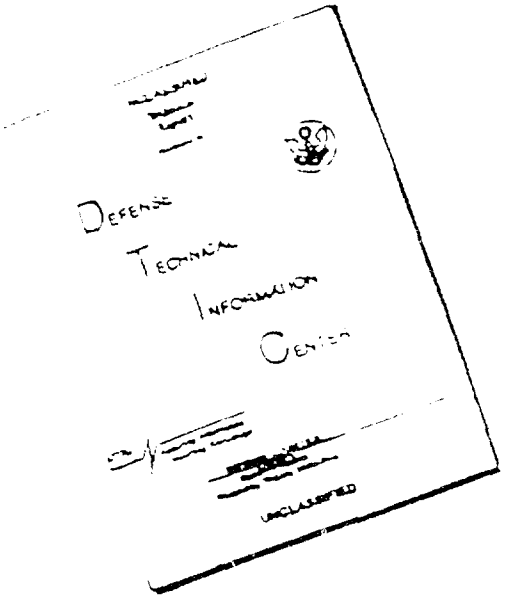
Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

Distribution Statement

Approved for public release; distribution is unlimited.

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) CRDEC-CR-007	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Pacific Northwest Laboratory 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) P. O. Box 999 Richland, WA 99352	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION CRDEC 8b. OFFICE SYMBOL (If applicable) SMCCR-RST-E	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DE-AC06-76-RLO 1830
8c. ADDRESS (City, State, and ZIP Code) Aberdeen Proving Ground, MD 21010-5423	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
Acute Environmental Toxicity and Persistence of a Chemical Agent Simulant: 2-Chloroethyl Ethyl Sulfide (CEES)

12. PERSONAL AUTHOR(S)
Cataldo, Dominic A., Ph.D.; Ligotke, Michael W., M.S.; (Continued on reverse)

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Contractor	FROM 86 Mar TO 87 Jan	1988 November	75

16. SUPPLEMENTARY NOTATION
COR: Dr. Randall S. Wentsel, SMCCR-RST-E, (301) 671-2036

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP
15	06	03
06	06	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Environmental persistence, Environmental effects
Vegetation surfaces, Plants, (JRC)
Soil surface
(continued on reverse)

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This report provides detailed information regarding a series of laboratory environmental fate and effects tests that were performed with the chemical agent simulant, 2-chloroethyl ethyl sulfide (CEES).

Aerosols of CEES were generated within a Henderson chamber to allow exposure of plants, soils, and soil microbial communities. Additionally, aerosols were generated for exposure of freshwater aquatic organisms. Aerosols were characterized for mass concentration and particle size with chemical content of the aerosols being determined by GC/MS.

Results for both aerosolized and surface-deposited CEES indicate that its vapor pressure is high enough to result in mixed gas and liquid phases. These affect the overall rate of deposition to surfaces. In addition, the volatility and rates of chemical decomposition

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL SANDRA J. JOHNSON	22b. TELEPHONE (Include Area Code) (301) 671-2914 22c. OFFICE SYMBOL SMCCR-SPS-T

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

12. Personal Authors (Continued)

McVeety, Bruce D., Ph.D.; Bolton, Harvey, Jr., Ph.D.; Fellows, Robert J., Ph.D.; Li, Shu-mei W., M.S.; Van Voris, Peter, Ph.D.; Crecelius, Eric A., Ph.D.; Hardy, Jack T., Ph.D. (Pacific Northwest Laboratory); and Wentsel, Randall S., Ph.D. (CRDEC)

18. Subject Terms (Continued)

Soil microbial Algae

19. Abstract (Continued)

of CEES appear to result in substantial loss of the chemical from foliar surfaces following application. The half-lives of CEES and HEES were found to be 2 to 5 and 4 to 8 h, respectively, following deposition to foliar surfaces. The half-life of CEES in soils was found to be 0.9 to 2.4 h. No measurable decline in concentration of HEES was observed over the 96-h treatment period. No VES was found associated with soil or foliar extracts.

The phytotoxicity of CEES is plant species dependent, with pine and sagebrush being most sensitive, and grass most tolerant. The simulant appeared to have a contact toxicity and did not seem to affect the onset or rate of new growth unless the initial damage was too severe. Metabolic studies showed an inhibition of photosynthetic capability and elevated respiration rates. Within the photosynthetic apparatus in the chloroplasts, those compounds of the electron transport chain closest to the outside of the thylakoid membrane (PS 1 and associated carriers) were the first to be affected. Results from in vitro testing of CEES indicated that concentrations below 10 µg/g dry soil generally did not immediately impact microbial activity in soil. Toxicity tests using Chlorella and Selenastrum species indicated that a surface dose equivalent to 20 g/m^2 is not likely to result in significant toxicity to freshwater algae.

PREFACE

The work described in this report was authorized under Contract No. DE-AC06-76-RLO 1830. This work was started in March 1986 and completed in January 1987.

The use of trade names or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This document has been approved for release to the public.

Acknowledgments

The authors wish to thank Don C. Klopher, Barbara A. O'Brian, and Bobbie Webster for technical support during this project. In addition, we would like to thank Dalene Bjazevich and Rosemarie Bartlett for their secretarial support, and Donna Brown, Steve Weiss, and Mindy Strong for their editing. Finally, our sincere thanks are extended to Dr. Wentsel, our technical monitor for this project.

Blank

CONTENTS

Page

1. INTRODUCTION 9
   1.1 Chemical Properties 11
   1.2 Environmental Fate and Effects 11
   1.3 Objectives 11

2. MATERIALS AND METHODS 13
   2.1 Exposure System 13
   2.2 Exposure Protocol 15
   2.3 Aerosol Generation 16
   2.4 Aerosol Characterization 17
      2.4.1 Predicted Aerosol Concentrations 17
      2.4.2 Bubbler Samples 18
      2.4.3 Syringe Samples 18
      2.4.4 Deposition Coupons 18
   2.5 Estimation of Dose to Plant and Soil Surfaces 19
   2.6 Chemical Analysis 19
   2.7 Plant Effects 21
      2.7.1 Gross Phytotoxicity 21
      2.7.2 Metabolic Effects 21
   2.8 Soil Microbial Assays 25
   2.9 Soil Characteristics 26
   2.10 Aquatic Assays 26
   2.11 Aquatic and Terrestrial Persistence 27
      2.11.1 Aquatic Stability and Fate 27
      2.11.2 Terrestrial Stability and Fate 28

3. RESULTS AND DISCUSSION 29
   3.1 Aerosol Characterization 29
      3.1.1 Predicted Aerosol Concentration 29
      3.1.2 Bubbler Samples 33
      3.1.3 Syringe Samples 33
      3.1.4 Deposition Coupons 33
      3.1.5 Summary of Aerosol Concentration Results 34
   3.2 Mass Loading on and Deposition Velocities to Foliar Surfaces and Soils 36
      3.2.1 Vegetative Surfaces 37
      3.2.2 Soil Surfaces 38
      3.2.3 Aerosol Deposition Velocities for Plants and Soils 39

Page

3.3 Phytotoxicity 41
3.3.1 Gross Phytotoxicity 41
3.3.2 Metabolic Effects on Plants 43
3.4 Effects on Soil Microbes 49
3.5 Terrestrial Persistence 54
3.5.1 Persistence in Soil 54
3.5.2 Persistence on Foliar Surfaces 54
3.6 Aquatic Behavior 58
3.6.1 Chemical Fate and Stability in Aquatic Systems 59
3.6.2 Effects on Phytoplankton 59

4. CONCLUSIONS 63

REFERENCES 65

APPENDIX A - QUALITY ASSURANCE AND QUALITY CONTROL 67

APPENDIX B - EXPERIMENTAL PROTOCOLS 71

FIGURES

Page

2.1 Henderson Chamber for Agent Simulant Exposure Tests 14

2.2 Analysis of Sagebrush Sample 1 Hour After Exposure 22

2.3 Analysis of Burbank Soil Sample Immediately After Exposure 23

3.1 Predicted CEES Aerosol Concentrations Exiting the Exposure Region of the Henderson Chamber During Tests CEES-01 and CEES-02 30

3.2 Predicted CEES Aerosol Concentrations Exiting the Exposure Region of the Henderson Chamber During Tests CEES-03 and CEES-04 31

3.3 Predicted CEES Aerosol Concentrations Exiting the Exposure Region of the Henderson Chamber During Test CEES-03 32

Page

3.4 Photosynthesis and Dark Respiration in µMol O_2/h/g dry wt of Isolated Leaf Segments from Sagebrush Before and After Exposure to CEES 44

3.5 Photosynthesis and Dark Respiration in µMol O_2/h/g dry wt of Isolated Leaf Segments from Short Needle Pine Before and After Exposure to CEES 45

3.6 Photosynthesis and Dark Respiration (O_2 uptake) in µMol O_2/h/g dry wt of Isolated Leaf Segments From Tall Fescue Before and After Exposure to CEES 46

3.7 Influence of CEES Soil Concentrations on Dehydrogenase Activity in Burbank and Palouse Soils 51

3.8 Influence of CEES Soil Concentrations on Phosphatase Activity in Burbank and Palouse Soils 53

3.9 Persistence of CEES and HEES in Maxey Flats Soil. 55

3.10 Persistence of CEES and HEES in Burbank Soil. 56

3.11 Residence Time of CEES and HEES on Foliar Surfaces of Sagebrush. . . 57

3.12 Residence Time of CEES and HEES on Foliar Surfaces of Short Needle Pine. 57

3.13 Residence Time of CEES and HEES on Foliar Surfaces of Tall Fescue. 58

3.14 Effects of CEES/HEES on Growth of Chlorella and Selenastrum. 62

TABLES

Page

1.1 List of Chemical Agent Simulants and Decontaminants Currently in Use by the U.S. Army. 10

2.1 Test Conditions During CEES Exposure Tests. 16

Page

2.2 Aerosol Generation During CEES Tests 18

2.3 Physical Characteristics of Soils. 27

3.1 Predicted Aerosol Mass Concentration During CEES Tests. 32

3.2 Aerosol Mass Concentration Determined by Analysis of Syringe Samples. 34

3.3 Mass Loading on Coupon During CEES Deposition Tests. 35

3.4 Aerosol Mass Concentration Results for CEES Tests. 35

3.5 Mass Loading of CEES, HEES and VES on Plant Surfa 38

3.6 Mass Loading of VES, CEES, and HEES on Soil Surfaces 39

3.7 Net Deposition Velocity of CEES to Plant and Soil Surfaces. 40

3.8 Coding for Modified Daubenmire Rating Scale and Associated Phytotoxicity Symptoms. 42

3.9 Phenotypic Responses of Several Plant Species Following Foliar Exposure to CEES 42

3.10 Interaction of CEES with Electron Transport Systems of Isolated Spinach Chloroplasts. 49

3.11 Effect of CEES on Dehydrogenase Activities in Burbank and Palouse Soils 52

3.12 The Effect of CEES on Phospha e Activities in Burbank and Palouse Soils 52

3.13 Effect of CEES/HEES on Growth Rates and Final Yield of Chlorella. 60

3.14 Effect of CEES/HEES on Growth Rates and Final Yield of Selenastrum. 61

ACUTE ENVIRONMENTAL TOXICITY AND PERSISTENCE
OF A CHEMICAL AGENT SIMULANT:
2-CHLOROETHYL ETHYL SULFIDE (CEES)

1. INTRODUCTION

Chemical simulants are substances whose characteristics partially resemble selected physical and chemical properties of chemical agents. Simulants are used in testing or trialing to determine the performance of equipment. Simulants are also used in training troops to operate equipment and to perform in a chemically contaminated environment.

Army Regulation (AR) 200-2 integrates environmental considerations into Army plans and programs. As implemented by the U.S. Army Materiel Command, this regulation requires environmental analyses and documentation for all items developed by subordinate research and development commands (Bennett 1984 [personal communication]). CRDEC as the developer is responsible for conducting research to generate environmental data on simulants. The purpose of this research effort is to conduct studies to determine the environmental fate and effects of simulants in terrestrial and aquatic systems. These data will support environmental issues on simulant use in testing, trialing, and training.

The CRDEC Environmental Fate and Effects Data Base is a compilation of physical, chemical, toxicological and environmental data of selected simulant agents. This data base also contains available data on chemicals employed for decontamination of simulant and agent contaminated equipment. The computerized data base can be accessed through the CRDEC Data Management Office. Reinbold et al. (1986) used this data base to conduct a hazard ranking of simulants. Based on this study, simulants were identified that required laboratory data to determine their environmental persistence and acute terrestrial and aquatic toxicity. Based on the rankings on Reinbold et al. (1986) simulants were selected from those shown in Table 1.1 that required further laboratory data to determine their environmental persistence and acute terrestrial and aquatic toxicity.

The use of chemical agent simulants and decontaminants at both U.S. and foreign training sites has the potential for producing significant environmental impacts. Only limited data on the chemistry and behavior of these materials are available (Bennett 1984 [personal communication]).

This report is one of a series, and it addresses just one of the chemical agent simulants listed in Table 1.1, namely 2-chloroethyl ethyl sulfide (CEES).

TABLE 1.1. List of Chemical Agent Simulants (CAS) and Decontaminants Currently in Use by the U.S. Army

Chemical Materiel	CAS Number
Simulant Agents	
Bis (2-ethylhexyl) 2-ethylhexyl phosphonate	126-63-6
Bis (2-ethylhexyl) phosphonate (BIS)	3658-48-8
n-Butyl mercaptan (BUSH)	109-79-5
2-Chloroethyl ethyl sulfide (CEES)	693-07-2
Diethyl adipate	141-28-6
Di (2-ethylhexyl) phthalate (DOP)	117-81-7
Diethyl hydrogen phosphonate (DEHP)	762-04-9
Diethyl malonate (DEM)	105-53-3
Diethyl phthalate (DEP)	84-66-2
Diethyl pimelate	2050-20-6
Diethyl sebacate (DES)	110-40-7
Diisopropyl fluorophosphate (DFP)	55-91-4
Diisopropyl methylphosphonate (DIMP)	1445-75-6
Dimethyl adipate (DMA)	627-93-0
Dimethyl hydrogen phosphonate (DMHP)	868-85-9
Dimethyl methylphosphonate (DMMP)	756-79-6
Diprophylene glycol monomethyl ether (DPGME)	34590-94-8
Ethanol	64-17-5
Ethyl chloroacetate (ECA)	105-39-5
Biethyl mercaptosuccinate, 0,0-dimethyl phosphorodithioate (Malathion)	121-75-5
Methyl salicylate	119-36-8
Diethyl p-nitrophenyl phosphate (Paraoxon)	311-45-5
Diethyl p-nitrophenyl thiophosphate (Parathion)	56-38-2
Polyethylene glycol 200 (PEG 200)	25322-68-3
Triethyl phosphate (TEP)	78-40-0
Trimethyl phosphate (TMP)	512-56-1
Decontaminants	
Diethylenetriamine	111-40-0
Perchloroethylene	127-18-4
Phenol	108-95-2
Chlorobenzene	108-90-7

The objective of these studies is to determine the potential acute environmental effects and persistence of various classes of agent simulants.

A previous report (Van Voris et al. 1987) described the environmental effects and chemical fate of two of these simulants, diisopropyl methylphosphonate (DIMP) and diisopropyl fluorophosphate (DFP).

1.1 CHEMICAL PROPERTIES

The simulant CEES is classed as a vesicant or blister agent, and has chemical similarities to mustards. While a relatively limited data base exists on the fate and effects of CEES, pertinent information concerning the chemical characteristics, stability, and toxicity of CEES has been reviewed elsewhere (Bennett, 1984 [personal communication]).

This simulant, the chemical formula for which is $ClCH_2CH_2SCH_2CH_3$, is a monochloro-dialkyl organosulfur compound with vesicant properties similar to but much weaker than mustard. It is liquid at room temperature and is partially soluble in water (approximately 1.7 g/L). Its important physical properties include a boiling point of 156°C and a vapor pressure of 3.4 mm Hg at 25°C. The compound is chemically unstable, reacts with a variety of matrices, and is particularly prone to hydrolysis in aqueous environments.

1.2 ENVIRONMENTAL FATE AND EFFECTS

The environmental persistence of CEES is dependent on its hydrolysis half-life, which in turn appears to be somewhat dependent on the pH of the waters, decreasing with increasing pH. Rapid hydrolytic attack of the Cl and S groups occurs in aqueous systems, resulting in a range of decomposition products. However, no reliable data are available for persistence in either terrestrial or aquatic environment.

1.3 OBJECTIVES

The purpose of these studies is to provide information about the environmental behavior and effects of agent simulants such as CEES. The scope of these efforts is restricted to assessment of impacts on a limited number of terrestrial and aquatic organisms based on contact toxicity and the chemical persistence of the simulant in soils and waters and on vegetative surfaces. These studies include only cursory evaluation of major decomposition products.

Blank

2. MATERIALS AND METHODS

The following methodology and experimental protocols were employed in evaluating the environmental effects and chemical fate of CEES. Terrestrial exposure studies were performed under dynamic exposure conditions using a Henderson chamber. This allowed for exposure of soils and plants, control and quantification of air concentrations, and calculation of deposition velocities. Studies of the persistence of CEES in soils and waters were conducted following in vitro amendment; this was necessary because of the short half-life of CEES and the low doses obtained via aerosol deposition. Aquatic studies were conducted using amendment methods.

2.1 EXPOSURE SYSTEM

All exposures of terrestrial plants and soils to CEES aerosols were performed in a Hendersen chamber at the Pacific Northwest Laboratory (PNL) Aerosol Research Facility. The test system was similar to that used previously for DIMP and DFP tests in 1985 (Van Voris et al. 1987). However, in order to provide better uniformity of aerosol concentrations, the flow rate through the system was increased approximately five times during the third and fourth CEES tests.

The exposure chamber, shown in Figure 2.1, contained the exposure of plants and soils to CEES aerosols. The exposure region of the chamber was 0.40 m^2 with a height of 0.6 to 0.9 m. Chamber volume was 0.37 m^3. A small fan was used in the exposure region to provide low-speed horizontal air motion (U^{max} less than ~1 m/s) and uniform mixing of CEES aerosols. An incandescent lamp was operated outside one of two large glass windows during all tests.

The exposure chamber was operated at negative air pressures of 2 to 15 cm- H_2O during test and purge periods to contain the CEES within the exposure system and to provide known air and chemical transfer rates. Fresh air was cleaned with high-efficiency particulate (HEPA) and carbon filters, mixed with CEES aerosol in a generation chamber, and passed into the bottom of the exposure chamber. Exhaust was drawn from the top of the chamber, passed through a dual-stage liquid scrubber and filters, and then pumped to the HEPA-filtered facility exhaust.

Uniformity of aerosol concentration within the exposure region of the test system was verified during trial tests. Glass fiber filter deposition coupons (47 mm) were suspended on springs at various locations in the exposure chamber at elevations representative of the plant canopy. The deposition

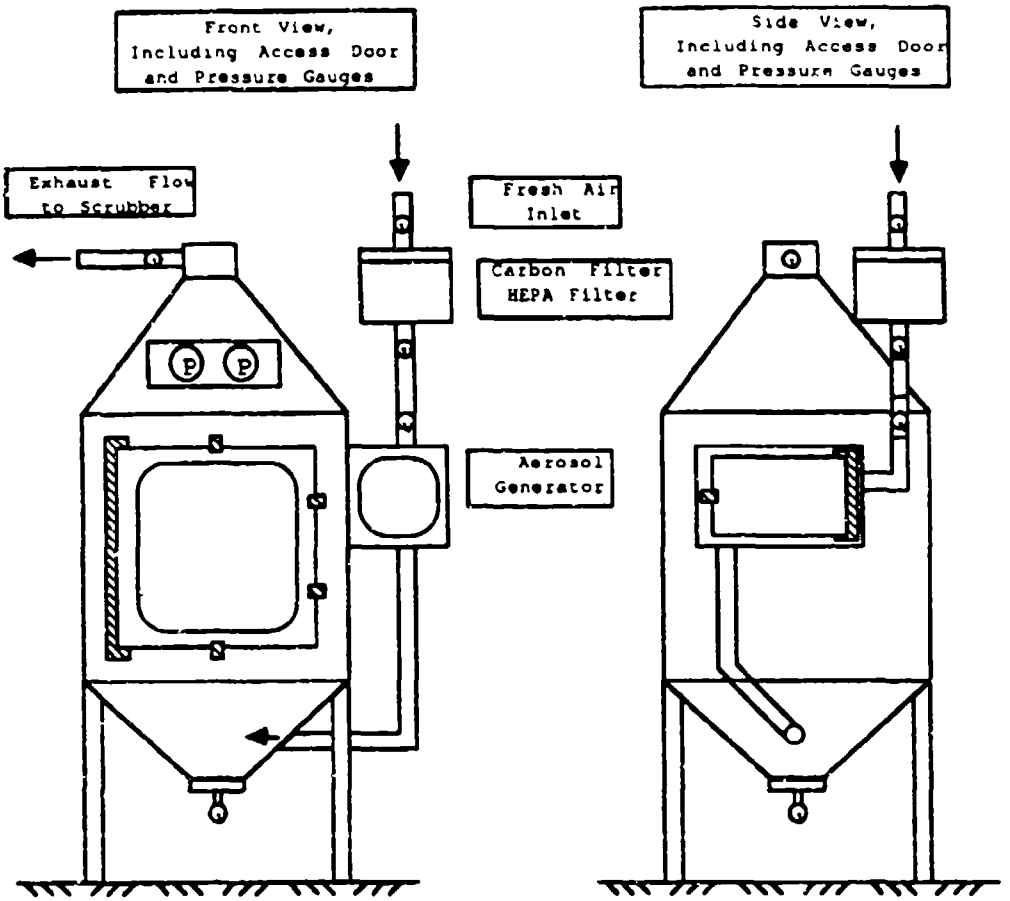


FIGURE 2.1. Henderson Chamber for Agent Simulant Exposure Tests

coupons were then exposed to an aerosol of oleic acid, generated using a nebulizer. The mixing fan was operated during the deposition test. Deposition to filters was uniform within ± 3.5% (1 ∂, n=9). In addition, it was observed that deposition to the bottom of the coupons accounted for only 2 to 7% of the total particle load. Similar partitioning of top and bottom loading during CEES tests was not anticipated for several reasons; particle size was likely smaller due to the relatively high vapor pressure of CEES (resulting in lesser importance of gravitational settling) as a mechanism of deposition, and deposition of the gaseous fraction of the CEES aerosols was likely controlled by plant surface sorptive processes. The volatility of CEES prevented any meaningful acquisition of particle size distributions.

2.2 EXPOSURE PROTOCOL

Four CEES exposure tests were completed for terrestrial plants and soils. Two tests (CEES-01 and CEES-02) were performed in August, 1986, and two (CEES-03 and CEES-04) were performed in August, 1987. Target aerosol concentrations were 100 mg/m^3 for CEES-01 and -03, and 1000 mg/m^3 for CEES-02 and -04.

Plants, soils, and deposition coupons were placed into the exposure chamber just prior to each test. Approximately 50% of the porous chamber floor was left uncovered to allow free transfer of the CEES aerosols into the exposure region. The deposition coupons were suspended horizontally within the plant canopy. Access doors on the exposure and generator chambers were sealed with grease and clamped shut. The lamp was turned on and air flows were initiated. The duration of each test was typically 60 min, followed by a 30-min purge. Effects of CEES on microbes in soil were studied by in vitro amendment of CEES to soils.

Aerosol generation procedures were determined by approximate methods prior to the tests. These methods depended on target concentration levels and on the air transfer rates within the exposure system. Samples were obtained during the tests to characterize aerosol concentration. These included grab samples by gas sampling syringes, and continuous samples by two bubblers in series. Samples were collected in distilled, deionized water (CEES-01 and -02) or GC grade hexane (CEES-03 and -04).

After completion of the purge period, plants were removed from the chamber and bagged to prevent contamination from other than foliar deposition. Sampling was begun, immediately after which plants were maintained in growth chambers and sampled periodically. Soil samples were removed, bagged, and made available for various analyses. Details of these procedures are described below.

After each set of tests, the exposure system was decontaminated by contact of all surfaces with copious amounts of 0.5 N NaOH. Components of the aerosol generation system were immersed in NaOH. The exposure system was then rinsed with water and allowed to dry. No chamber cleaning was performed between low- and high-concentration tests because the possibility of residual contamination was minimal and the tests were performed on the same day.

Test conditions are listed in Table 2.1. Flow rates in the system during the tests were determined as the sum of the inlet flow, the volume of air delivered by the nebulizer, and the measured volume of leaking air into the exposure chamber. This leak was less than ~5% of the total flow during the

TABLE 2.1. Test Conditions During CEES Exposure Tests

Test	Date	Temp. (°C)	RH (%)	Duration		Flow Rate	
				Test (min)	Purge (min)	Test (L/min)	Purge (L/min)
CEES-01	8/26/87	ND[a]	ND	60	15	14	100
CEES-02	8/26/87	ND	ND	60	30	14	100
CEES-03	8/17/87	21 ±2	38 ±5	60	30	64	81
CEES-04	8/17/87	21 ±2	38 ±5	34	30	69	63

[a] ND = No computer data recorded; temperature and relative humidity estimated at 21 ± 2°C and 35 ± 15%, respectively, based on recorded values for tests preceding and following these tests.

tests and was primarily caused by negative air pressure on the access door seals. Increased flow rates were maintained during most chamber-purge cycles.

2.3 AEROSOL GENERATION

Aerosols of CEES were generated intermittently or continuously during tests by operation of an ABCO No. 535092 polyethylene nebulizer at a pressure of 10 psi. The 25-ml reservoir of the nebulizer was filled with neat CEES. The nebulizer was located in an aerosol generation chamber attached to the exposure chamber and monitored through a window during tests.

A nebulizer was chosen to generate agent simulant aerosols for exposure tests because of its capability to generate micrometer-sized droplets and to maintain uniform output of neat liquid aerosols for extended periods. In addition, use of a nebulizer allowed the rapid on/off switching suitable for intermittent generation procedures. In a nebulizer, aerosols are produced by aspirating liquid from a reservoir with a high-velocity air jet. The liquid is deposited as a film over an obstruction, such as a small sphere. The liquid film separates from the sphere and is atomized in the shear zone of the air jet. Large particles are trapped on the surfaces of the nebulizer outlet and drain into the reservoir; small particles escape and are entrained in the inlet flow of fresh air to the exposure chamber.

Operation of the nebulizer was continuous during one high-dose test and intermittent during one high-dose and two low-dose tests. The nebulizer was operated intermittently by connecting and disconnecting a supply of 10 psi air.

Periods of intermittent generation included 6 s of nebulizer operation during 1- to 5-min intervals and 56 s of operation during 2- to 5-min intervals. Rate of aerosol generation by nebulizers is a function of liquid properties and varies among chemicals. The CEES aerosol was observed to be generated at a rate of 0.40 ± 0.03 ml/min. Sources of errors in measurement during the intermittent generation periods included < 0.5 s of uncertainty for each 6 s of generation and were estimated to be limited to 5%. Details of aerosol generation for all CEES tests are listed in Table 2.2. Generation ceased after 34 min during CEES-04 because the reservoir had been depleted. This was because of the greater-than-expected volumetric generation rate (0.40 ml/min versus 0.15 ml/min).

The rate of generation of CEES in the nebulizer was observed to be greater than that for the other simulant materials tested to date (DIMP and DFP). In addition, a large fraction of the aerosolized CEES likely volatilized, resulting in gas-phase rather than liquid-phase droplets. A haze of liquid droplet aerosol was visible in the generator and the exposure chamber during all non-CEES tests; however, the only location of visible CEES aerosol was at the immediate exit of the nebulizer. These observations were expected based on the higher of two CEES vapor pressures discussed in open literature. With a vapor pressure of 3.4 mm-Hg, CEES should be much more volatile than DIMP (0.17 mm-Hg), or DFP (0.58 mm-Hg).

2.4 AEROSOL CHARACTERIZATION

Procedures for aerosol generation were determined for target aerosol mass concentrations of 100 and 1000 mg/m^3. Because no trial tests were performed, attainment of these targets was difficult. Measurements of aerosol concentration and deposition were made during each test. These included bubbler samples, syringe (grab) samples, and deposition coupons.

2.4.1 Predicted Aerosol Concentrations

Aerosol concentrations were estimated by comparing volumetric rate of chemical generation to system volume and flow rates. Rate of generation was estimated to be 0.15 ml/min--the actual rate was later measured to be 0.4 ml/min. Concentration losses due to generation inefficiencies and deposition and adsorption to chamber, plant, and soil surfaces were unknown and could not be included accurately in concentration estimates prior to the tests. In an attempt to circumvent these losses, the actual volume of CEES generated was 2-3 times that estimated to be required. Because losses were minimal and the actual generation rate was greater than assumed, the resulting CEES concentrations were greater than those targeted.

TABLE 2.2. Aerosol Generation During CEES Tests

Test	Dose	Period[a] (on/off)	Time On[b] (min)	Σ(Volume) (ml)
CEES-01	Low	6/5	1.2	0.48 ± 0.06
CEES-02	High	56/2-5	12.2	4.92 ± 0.37
CEES-03	Low	6/1	6.0	2.41 ± 0.31
CEES-04	High	Continuous	34.0	13.67 ± 1.02

[a] Generator cycle time defined as s on/off interval in minutes.
[b] Total time generator was on during exposure.

2.4.2 Bubbler Samples

Two glass bubblers were used in series to obtain a time-averaged sample of the aerosol mass concentration in the exposure chamber. The bubblers were typically operated throughout the duration of the test period, but not during the purge. Samples at 0.5 or 1.0 L/min were passed through deionized water or hexane in Ace Glass No. 7529 smog bubblers. Porosity C (25 -50 μm) fritted bubbler tips were used. Samples were collected in distilled, deionized water (CEES-01 and -02) or GC grade hexane (CEES-03 and -04). The initial volume of solvent was 50.0 ml; however, significant evaporation (up to ~50%) occurred during some sampling periods when hexane was used.

2.4.3 Syringe Samples

Four grab samples were obtained from the exposure chamber during each test to provide another measure of aerosol mass concentration. Aerosol samples of 50, 60, and 80 ml were drawn through a 7-inch-long BD20 stainless steel needle into a 100-ml glass syringe. Approximately 10 ml of hexane was then drawn into the syringe, and the sample was washed for 2 min. The solvent was then returned to its original vial, resulting in 10.0 ml of total solution. The gas-washing procedure was repeated for samples obtained during CEES-04 to determine the efficiency of collection and removal of CEES from the syringe trap.

2.4.4 Deposition Coupons

Four or six deposition coupons were suspended within the plant canopy in the exposure chamber during each test. The coupons were 47-mm glass-fiber filters (Gelman Type A/E) and were suspended horizontally using spring holders.

The total area available for deposition, both top and bottom, was 34.0 cm^2. The coupons were removed from the chamber at the end of the purge period and contacted with hexane. During CEES-04, coupon masses were obtained before and after the exposure.

2.5 ESTIMATION OF DOSE TO PLANT AND SOIL SURFACES

Leaf tissue (duplicate samples from different places within the canopy) contaminated with CEES were placed in glass vials containing 10 ml of high-purity, distilled-in-glass hexane and extracted for 10 min. The vials were fitted with Teflon-lined screw caps. Following extraction, the tissues were removed from the vials and leaf areas were measured using a Licor LI-3000 leaf area meter. The foliar mass loading was calculated as ng contaminant/cm^2 leaf surface.

Three subsamples of each soil sample were removed from Petri dishes exposed to aerosols using a cork borer (sample area was 3 x 0.95 cm^2), and the samples were placed into a 25-ml tared Corex centrifuge tube with a Teflon-lined screw cap. Five ml of hexane were added to the soil sample, and the tube was vigorously shaken for 1 min before the solid and liquid phases were separated. Soil samples were centrifuged at 8000 x g for 10 min at 25°C, using a Beckman model J2-21M centrifuge. The hexane was transferred to a clean vial, and the soil samples were air dried for 16 h and dried at 60°C for 8 h. Tared tubes were reweighed to obtain the dry weight of the soil and mass loading was calculated (ng contaminant/g soil). All sample extracts from the tests were kept frozen at -80°C before analysis.

These same extraction procedures were employed for all four CEES exposure tests, except that during CEES-1 and -2, water was used as the extraction solvent. The aqueous extraction of plant tissues and soil samples resulted in the complete conversion of the spiked CEES to its hydrolysis product HEES (ethyl 2-hydroxethylsulfide), which was subsequently derivatized and quantified. Using this method, the recovery of CEES (as HEES) from spiked plant tissues and soil samples was 74% ± 7%. Using hexane as the extraction solvent allowed the separate identification of the parent compound CEES and its decomposition products, HEES and VES (vinyl ethylsulfide). Their degrees of recovery averaged 81% ± 9% from spiked plant tissue and 76±6% from soils. Although these degrees of recovery might have improved slightly through exhaustive soxhlet extraction, the benefits of any additional recovery would be offset by increases in sample handling time and associated chemical changes.

2.6 CHEMICAL ANALYSIS

As stated above, two different analytical techniques were employed for

the quantification of CEES residue on plant and soil surfaces. For samples collected during CEES-1 and CEES-2, the aqueous extracts were derivativized using chloramine-B (sodium benzenesulfochloramide), after which reverse phase HPLC analysis was conducted using UV detection. This method, adapted from Bossle et al. (1983), was later abandoned because it failed to give necessary information on the relative ratio of CEES to HEES. Thus, the derivatization method was replaced by one developed at PNL using capillary gas chromatographic mass spectrometry (GC/MS).

The GC/MS method was conducted on a Hewlett-Packard 5880 capillary gas chromatograph coupled with a Hewlett-Packard 5970a mass selective detector. Hexane extracts of the tissues, soils, deposition coupons, and air samples were transferred to autosample vials and fitted with Teflon-lined crimp-top septa seals. These extracts were analyzed for CEES and its decomposition products without further manipulation.

The gas chromatograph was operated in the splitless injection mode with a loading time of 0.6 min. The column used was a 30-m fused silica capillary column with a polyethylene glycol liquid phase, cross linked and bonded with wax to the fused silica surface. The chromatograph oven was temperature-programmed from 25°C to 180°C at 8°C/min, with a 4-min hold at the initial temperature. At 180°C the oven temperature was programmed at 20°C/min to a final temperature of 250°C. The injection port and transfer line to the mass spectrometer were set at 250°C. The quadruple mass spectrometer was operated in the selective ion monitoring mode using a standard PFTBA tune.

The parent compound, CEES, and the two decomposition products, HEES and VES, were quantified with external standards. Three mass ions were selected for each compound. The criteria for selection was that they were major ions of significant abundance that were free of interferences. For CEES, mass to charge ratios (m/z) of 75, 124, and 126 were monitored with a dwell time of 50 milliseconds for each ion. For HEES, m/z 61, 75, and 106 were used, and for VES the selected ions were m/z 60, 73 and 88. A 6-point calibration curve was constructed for each compound, with a dynamic range covering three orders of magnitude. Each compound was run in triplicate during calibration, and the best-fit regression line was used to relate the integrated peak area to the mass of compound injected into the mass spectrometer. The detection limit for the three compounds in soil was approximately 10 ng/cm^2, and 1 ng/cm^2 on plant tissues. The difference in order of magnitude in detection limits was a function of the difference in sample size; in a typical sample, about 10 times more plant surface than soil surface was sampled.

Data acquired from typical samples of plants and soil are given in Figures 2.2 and 2.3. These figures show the three retention-time windows for VES,

CEES, HEES, respectively. Figure 2.2 is the mass chromatogram of a hexane extract taken from sagebrush vegetation 1 h after exposure, and Figure 2.3 is the mass chromatogram of an extract of Burbank soil immediately after exposure. The proper peaks are labeled in these figures. Note that there is a slight difference in the retention times of CEES and HEES between the two samples, a negligible difference of 3 to 7 s, which occurred over a 2-week period.

2.7 PLANT EFFECTS

The effects of CEES on vegetation were explored by studying evidence of phytotoxicity and metabolic effects in three species: the short needle pine (*Pinus echinata*), tall fescue (*Festuca arundinacea*, 'K-13') and sagebrush (*Artemisia tridentata, vaseyana*).

2.7.1 Gross Phytotoxicity

Assessments of phytotoxicity resulting from foliar contamination with CEES were based on the development of visual toxicity symptoms. These symptoms included leaf burn, blade die-back, necrotic spotting, and chlorosis. Quantitation of these effects is based on a Modified Daubenmire Rating Scale (MDRS) (Daubenmire 1959), a discussion of which is included in the results section of this report.

2.7.2 Metabolic Effects

The basis of the observed phytotoxicity of CEES on whole plants was further investigated using two *in vitro* systems: 1) the effects of the simulant on photosynthesis (oxygen evolution) and dark respiration (oxygen uptake) in intact leaf segments; and 2) the effects of the simulant on specific photochemical reactions and electron transport chains in isolated chloroplasts.

Whole Leaf Measurements

Leaf samples from the different species exposed at high and low concentrations of the chemical were taken prior to, immediately following, and at several intervals after exposure for analysis of oxygen evolution and uptake. Leaves were excised from the plants, placed in moistened paper towels, and maintained on ice at approximately 4°C until assayed. They were then wet with distilled water and sliced with a razor blade into pieces <5 mm in length or diameter. The pieces were transferred to an assay medium consisting of

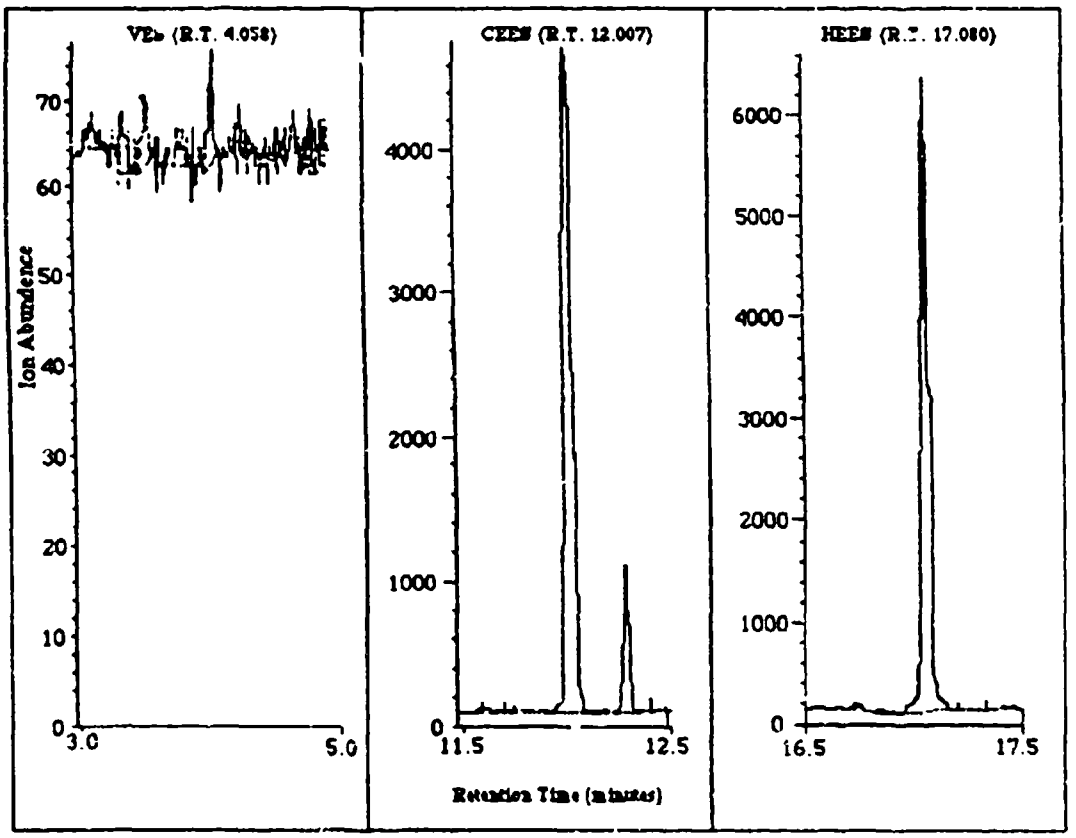


FIGURE 2.2. Analysis of Sagebrush Sample 1 Hour After Exposure. (Total ion chromatograms for retention times windows surrounding eluting peaks of VES, CEES, and HEES, respectively. For VES, plot represents the summed ion current for m/z 60, 73, and 88; for CEES, m/z 75, 124, and 126; for HEES, m/z 61, 75, 106. Note that the CEES to HEES ratios are close to one in this sample.)

2 mM $CaCl_2$, 10 mM sodium bicarbonate, and 20 mM N-2-Hydroxyethylpiperazine-N'-2-ethansulfonic acid (HEPES) pH 7.6. Paired tissue samples were taken from this solution and placed directly into paired, water-jacketed (3.9 ml of control media at 20±1°C) cuvettes. The suspension was continually stirred with magnetic stirrers. The cuvettes were then covered with aluminum foil for dark respiration for approximately 25 min, until a steady-state rate was obtained. They were then illuminated with saturating light (>1200 µEinsteins $m^{-2}s^{-1}$) at 600 nm for an additional 20 min to obtain a steady-state rate of photosynthesis. After illumination, the tissues were removed from the cuvettes, and blotted and dried overnight in a 75°C oven so the dry weight could be obtained. Assays were run in triplicate and the data expressed as µMol O_2 $h^{-1}g$ dry wt^{-1}.

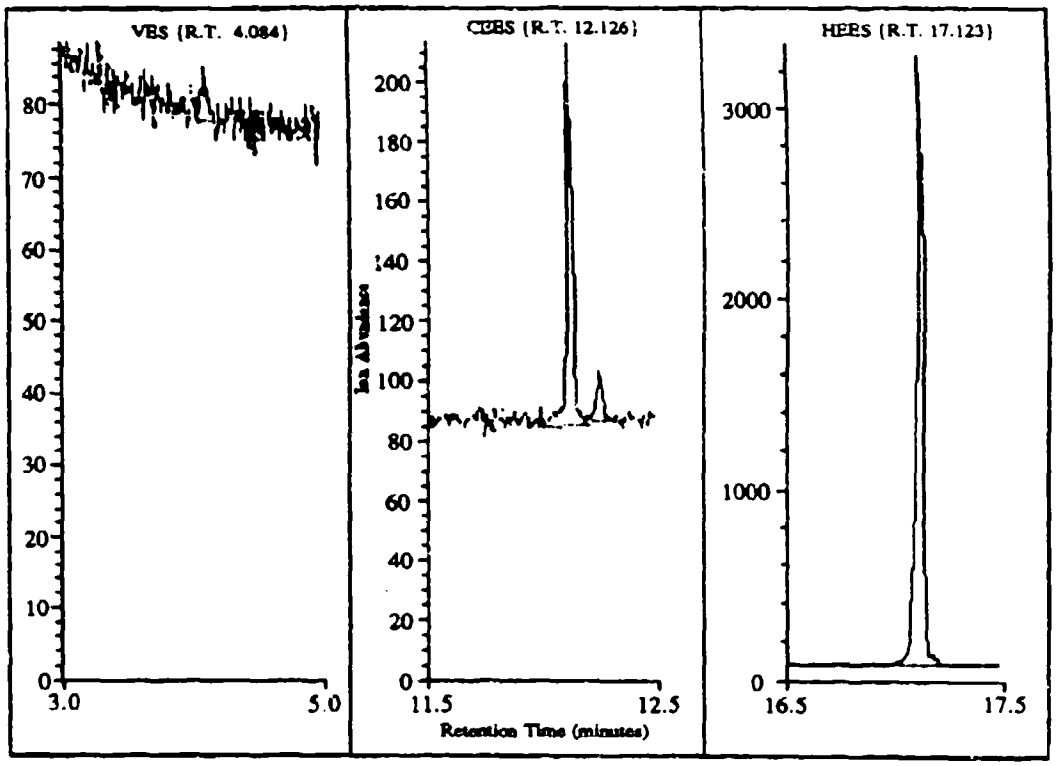


FIGURE 2.3. Analysis of Burbank Soil Sample Immediately After Exposure. (Total ion chromatograms for retention times windows surrounding eluting peaks of VES, CEES, and HEES, respectively. For VES, plot represents the summed ion current for m/z 60, 73, and 88; for CEES, m/z 75, 124, and 126; for HEES, m/z 61, 75, 108. Note the relative ratio of CEES to HEES in this sample. The CEES has been almost completely converted to the hydrolysis product, HEES.)

Isolated Chloroplast Measurements

Chloroplasts were isolated from commercially obtained spinach (*Spinacea oleracea*) leaves according to the methods of Walker (1980). Approximately 80 g of leaves with the mid-ribs removed were washed with distilled water and chilled prior to grinding. The leaves were then ground for 10 s with a sorvall tissue homogenizer in 50 ml of grinding medium, consisting of: 0.33 M Sorbitol; 10 mM $Na_4P_2O_7$; 5 mM $MgCl_2$; and 2 mM sodium ascorbate, pH 6.5 which had been chilled to a slush-like consistency to maintain the grinding temperature around 4°C. The ground material was then filtered through 8 layers of cheesecloth and the filtrate immediately centrifuged at 1500 x g for 90 s. The supernate was then decanted and the surface of the pellet washed with 1 ml of resuspension

mix which was then discarded. The pellet was resuspended in a mixture consisting of 0.3 M sorbitol; 2 mM Ethylenediaminetetraacetic Acid Disodium Salt (Na_2 EDTA);1 mM $MgCl_2$; 1 mM $MnCl_2$; 50 mM HEPES; 10 mM $NaHCO_3$; 5 mM $Na_2P_2O_7$ (PP_i); 0.5 mM Na_2HPO_4 (P_i), pH 7.6. Chlorophyll content was determined according to the method of Arnon (1949): 50 µl of the chloroplast suspension was added to 20 ml of 80% (v/v) acetone and filtered (through No.1 Whatman paper), and the absorbance read at 652 nm. Nine divided by the absorbance gives the volume of the original suspension containing 100 µg of chlorophyll. All procedures were carried out under low light and at 4±1°C.

Photochemical Assays

Assays of photosystems (PS) were conducted on PS II, PS I, and chloroplast whole-chain electron transport, measuring oxygen evolution and uptake with a Clark-type electrode (YSI Instruments) in a 1.8-ml volume, water-jacketed cuvette (Gilson Medical Electronics) maintained at 20±1°C. Stock solution of the CEES was prepared so that addition of 100 µl would equal a final concentration within the cuvette of 1 or 10 ppm. All assays were conducted in paired cuvettes at the same time, with one cuvette serving as a control and the other containing the simulant. The CEES was either added directly to the cuvette prior to illumination (~1 min) or to a chloroplast suspension in a test tube for 1 h prior to transfer to the cuvette for assay. Control chloroplasts were treated in the same manner. Assays were run in triplicate, and all data are expressed in either µMol O_2 $h^{-1}mg^{-1}$chl or as % control of the paired assay. The analyses of the three components of the chloroplast electron transport system were performed according to the following methods:

PS II Measurements. Assays were conducted according to the methods of Boyer and Bowen (1970). The assay medium (1.8 ml) consisted of 0.33 M sorbitol; 2 mM Na_2EDTA; 1 mM $MgCl_2$; 1 mM $MnCl_2$; and 50 mM HEPES, pH 7.6. Sodium 2,6-dichloroindophenol (DCIP), 0.98 mM, was added just prior to the addition of chloroplasts (100 µg). The suspension was then illuminated from the side with saturating light (>1200 µEinsteins $m^{-2}s^{-1}$) at 600 nm, and the rate of oxygen evolution determined from the initial slope of the electrode output as a function of time.

PS I Measurements. Assays were conducted according to the methods of Keck and Boyer (1974). The assay medium consisted of 1 mM ADP, 1 mM K_2HPO_4, 0.1 M KCl, 5 mM $MgCl_2$, 0.1 mM 3-(3,4-Dichlorophenyl)-1,1-Dimethylurea (DCMU), 80 µM DCIP, 1 mM sodium ascorbate, 0.5 mM methyl viologen (MV), 0.5 mM sodium azide (prepared daily), and 100 µg chlorophyll. Assays were illuminated and measured as above.

Whole-Chain (Water to MV) Measurements. Assay conditions were identical to those described for PS I measurements (Keck and Boyer 1974), except that DCMU, DCIP, and sodium ascorbate were deleted from the medium.

2.8 SOIL MICROBIAL ASSAYS

The effects of CEES on soil microbial and biochemical activities were evaluated *in vitro* by measuring the activity of two soil enzymes. Stock solutions of CEES (Aldrich Cat. No. 24264-0, Lot No. KM00903JM) were prepared in distilled water and added to samples of Palouse and Burbank soils (to final concentrations ranging from 0 to 250 µg/g dry soil) and incubated at 22°C in the dark.

Soil samples were assayed for dehydrogenase activity as described by Tabatabai (1982) immediately following incubation and after 1 week and 4 weeks. Soils amended with CEES (5 g dry weight basis) were first mixed with 0.015 g of $CaCO_3$. 0.3 ml of 1% glucose and 0.25 ml of 3% 2,3,5-triphenyltetrazolium chloride (TTC) and incubated for 24 h at 22°C. Ten ml of methanol was then added to the soil and mixed thoroughly. The mixture was centrifuged and the absorbance of the supernatant at 485 nm was measured using a Beckman DU-50 spectrometer. Soil dehydrogenase activity, expressed as mg of TTC-formazan produced per g of soil/24 h, was quantified by comparing absorbance values to a standard curve prepared with reagent-grade TTC-formazan and methanol.

Soil phosphatase activity was measured on the CEES-amended soil using the procedure described by Tabatabai and Bremner (1969) as modified by Klein et al. (1979). One g of soil (dry weight) was placed in 15-ml centrifuge tubes with 4 ml of modified universal buffer (MUB), which consists of tris(hydroxymethyl) amino methane, 3.025 g; maleic acid, 2.9 g; citric acid, 3.5 g; boric acid, 1.57 g; 1 N NaOH, 122 ml, yielding final volume of 250 ml pH 8.65. One ml of 0.025 N para-nitrophenol phosphate, prepared using MUB, was added to each tube. The tubes were stoppered, vortexed and incubated for 1 h at 37°C. One ml of 0.5 N $CaCl_2$ and 4 ml of 0.5 N NaOH were then added to stop the reaction. The mixtures were centrifuged at 12,000xg for 10 min, and absorbance of supernatant was measured at 400 nm with a spectrophotometer. Phosphatase activity was determined by comparing these values to a standard curve constructed with reagent-grade para-nitrophenol and expressed as µg of para-nitrophenol released per g of soil/hour.

All dehydrogenase and phosphatase activities were measured in duplicates and mean values were compared with those of the control soil (not CEES-treated) and expressed as percent of those of the control.

2.9 SOIL CHARACTERISTICS

Soils used for evaluation of simulant deposition efficiency, chemical transformations, depuration, and microbial metabolism effects were Burbank sand, Maxey Flats clay, and Palouse silt-loam. All soils were air-dried and sieved to pass a 2-mm screen. The physical characteristics of these soils are shown in Table 2.3. The Palouse is a highly productive agricultural soil in the dry-land wheat (*Triticum aestivum*) growing region of Washington State. Burbank sand is an arid soil located on the Hanford Reservation in Washington State. Maxey Flats is an infertile acidic clay.

2.10 AQUATIC ASSAYS

Cultures of the freshwater algae *Selenastrum capricornutum* and *Chlorella pyrenoidosa* were obtained from Carolina Biological Supply and maintained in a productive state in an incubator at 18°C with 18 h light per day. The nutrient growth medium used was Algo-Gro in water from Lake Crescent, a pristine lake on the North Olympic Peninsula, Washington.

Range-finding tests for CEES were conducted with concentrations of the sulfide agent over the range of 0 mg/L to 500 mg/L. An aqueous stock solution of sulfide was mixed at 1 g/L concentration and dilutions of 500, 250, 100, 10, and 1 mg/L were made in borosilicate glass culture tubes (25 mm x 150 mm) using growth medium as the diluent. To ensure that each culture tube contained an equivalent concentration of nutrients, a double-strength mixture of nutrient medium was mixed and used in proportion to the volume of CEES stock solution. Before additions of stock solution were made, culture tubes containing algal growth media were capped, pasteurized at 75°C for 4 h, and cooled to 18°C in an incubator. Tubes were inoculated with 1 ml of algal culture and then dosed with CEES stock solution. Final volume in the culture tubes ranged from 25 to 28 ml. Control samples contained no stock solution and received no algal inoculation.

Culture tubes were placed in a rack on a shaker table, which provided gentle agitation during the bioassay. Measurements of optical density of the liquid cultures were made daily at wavelength 670 nm using a Bausch and Lomb Spectronic 20, equipped with a wide-range phototube and appropriate filter (sensitive 400-700 nm). Before each measurement of optical density, contents of the tubes were homogenized on a vortex mixer. To compensate for possible inconsistencies in light intensity on the shaker table, positions of the culture tubes were shifted daily. A color blank containing green food dye was analyzed daily as a control for instrument accuracy over the test period.

TABLE 2.3. Physical Characteristics of Soils

Parameter	Soil Series		
	Burbank	Maxey Flats	Palouse
Textural Classification	sandy loam	clay	silt loam
Family	sandy, skeletal, mixed xeric		fine-silty, mixed mesic
Subgroup	Torriorthent		Pachic Ultic Haploxeroll
Location	Hanford, WA	Maxey Flats, KY	Pullman, WA
Sampling Depth (cm)	0-10	0-10	0-15
Horizon	A_p	A_1	A_p

Chlorella cultures tended to adhere to the sides of culture tubes and could not be readily shaken into uniform suspension. To correct for inaccuracies in optical density caused by this problem, culture tubes containing Chlorella were agitated twice daily and rolled 180 degrees in the rack to prevent excessive build-up on the surface of the tube. Readings of optical density were made from four sides (front, back, left, and right sides) of culture tubes, with the mean of these values recorded each day. Selenastrum cultures did not exhibit this tendency toward adhesion to the tube walls and thus required agitation only once a day.

Results from the range-finding tests helped define a narrower dilution range for the definitive tests. Methodology for definitive tests was unchanged. Both Selenastrum and Chlorella were cultured in a nutrient solution at 0, 100, 120, 140, 160, 180, and 200 mg/L CEES for the definitive tests.

Growth rate was determined by the optical density reading of the cultures during the logarithmic phase of growth (day 8 for Chlorella, day 4 for Selenastrum), and values of the treatments were expressed as percentages of the controls (algal cultures without added simulant). Final yield was defined as the optical density after growth had reached a stationary phase. The significance of differences between the optical densities of the treated cultures and those of the controls was evaluated using Student's t-test.

2.11 AQUATIC AND TERRESTRIAL PERSISTENCE

2.11.1 Aquatic Stability and Fate

Evaluations of the chemical behavior of CEES in aquatic systems were performed at the Battelle Marine Research Laboratory in Sequim, Washington.

Prior to exposure tests, several analytical approaches were evaluated. The CEES and HEES were obtained from Aldrich Chemical Company and VES from Alpha Chemical. Preliminary investigations suggested that GC-FID analysis would have several advantages over the HPLC used by Bossle et al. (1984), including excellent sensitivity, lack of need for a derivatization agent, and considerable cost savings. The CEES and HEES were extracted from water samples by solvent extraction into methylene chloride and analyzed by GC using a DB Carbowax capillary column and FID.

To determine aqueous stability, CEES was added to deionized water in both clear (light) and amber (non light) bottles at a concentration of 107 mg/L, capped tightly, and sampled immediately after 6 to 8 min, and after 1 h. The samples were then compared with a spike of CEES in solvent (methylene chloride).

Experiments on the behavior and fate of CEES applied to the water surface were conducted as follows: Two polycarbonate tanks were filled with 5 L each of water from Lake Crescent. A manifold provided a surface wind velocity of approximately 7 mph in tank A; tank B had no surface wind. Both tanks were covered with a domed lid provided with a small sampling port. The center of the tanks contained small platforms with 4 Gelman GF (A/E) filters (25 mm diameter) to measure the deposition to the surface. A control sample of the surface water was taken with a filter prior to introducing CEES. One ml of CEES solution (1075 mg) was sprayed (duration 45 s) with an air brush through the sampling port and allowed to settle on the water surface. The aquatic surface microlayer was sampled by floating 25-mm-diameter GF filters on the water for approximately 10 s, retrieving the filters with forceps, and placing them in glass vials with aluminum-lined caps (scintillation vials). Ten-ml samples of subsurface water (10 cm depth) were collected with a glass syringe. Samples were collected after 1 min and 1, 4, 24, 48, and 96 h.

Microlayer and water samples were extracted with 5 ml of methylene chloride and analyzed by GC. Spike-recovery experiments indicated that 69.3 ± 1.1% (s.d.) of the HEES was recovered from aqueous solution.

2.11.2 Terrestrial Stability and Fate

The terrestrial persistence of CEES and the extent of formation of HEES and VES were determined for soil surfaces and foliar surfaces exposed to aerosols of CEES. Samples were collected and analyzed within 1 h of exposure, and sampling continued until significant depletion of CEES occurred.

3. RESULTS AND DISCUSSION

Results from the CEES tests are described in the following sections.

3.1 AEROSOL CHARACTERIZATION

The chemical reactivity and volatility of CEES necessitated that measurements of air concentrations be performed by several methods. A comparison of the various sampling methods indicated that the bubbler samplers and deposition coupons were not as effective for quantification of CEES as the syringe samplers.

3.1.1 Predicted Aerosol Concentration

Calculated aerosol concentration histories were determined for each test based on actual characteristics of the exposure system and the actual volumetric rate of CEES generation during the tests (0.40 ml/min). (See Figures 3.1 through 3.3). The plots were calculated assuming that no loss of aerosol concentration occurred due to deposition and adsorption to chamber, plant, and soil surfaces. No chemical degradation or absorption of water vapor by CEES droplets was considered. Intermittent generation procedures were converted to equivalent generation rates and durations for 1-min periods (the actual test procedure used during CEES-03) to simplify calculations. Potential errors in the measured CEES generation rate were ± 0.05 ml/min during CEES-01, -02, and -03, and ± 0.03 ml/min during CEES-04 (see Figures 3.1 and 3.2). Because the nebulizer was operated intermittently during all tests except CEES-04, actual aerosol concentrations fluctuated slightly during test periods. These fluctuations were somewhat greater during CEES-03 because of a higher rate of air transfer within the exposure system. (For clarity, Figure 3.2 shows CEES-03 without aerosol fluctuations.) The predicted concentration history for CEES-03, including the intermittent generation of aerosol, is shown in Figure 3.3.
The importance of these calibration plots is discussed below.

The predicted average aerosol mass concentrations for each test plus purge are listed in Table 3.1. The predicted rate of generation, 0.40 ml/min, was used in the calculations. Errors reflect uncertainty in volumetric aerosol generation rate. By including a loss factor in the mass concentration calculations, it would be possible to reduce the calculated aerosol mass concentration for losses of chemical as a result of inefficiencies of aerosol generation and deposition and adsorption of the chemical to plant, soil, and other surfaces in the exposure system.

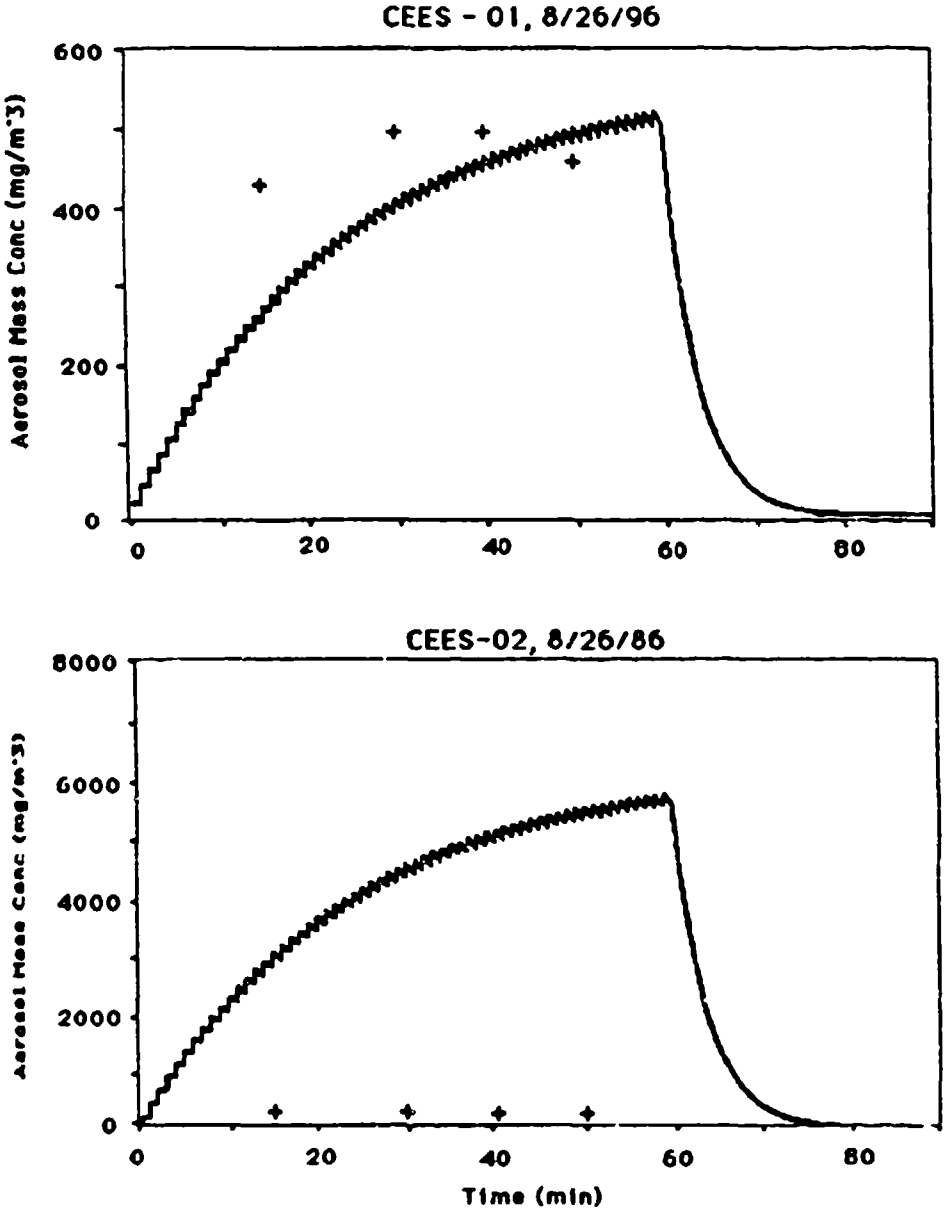


FIGURE 3.1. Predicted CEES Aerosol Concentrations Exiting the Exposure Region of the Henderson Chamber During Tests CEES-01 and CEES-02. Fluctuations represent the influence of intermittent aerosol generation. Data points on the plots represent CEES air concentrations determined from syringe grab samples.

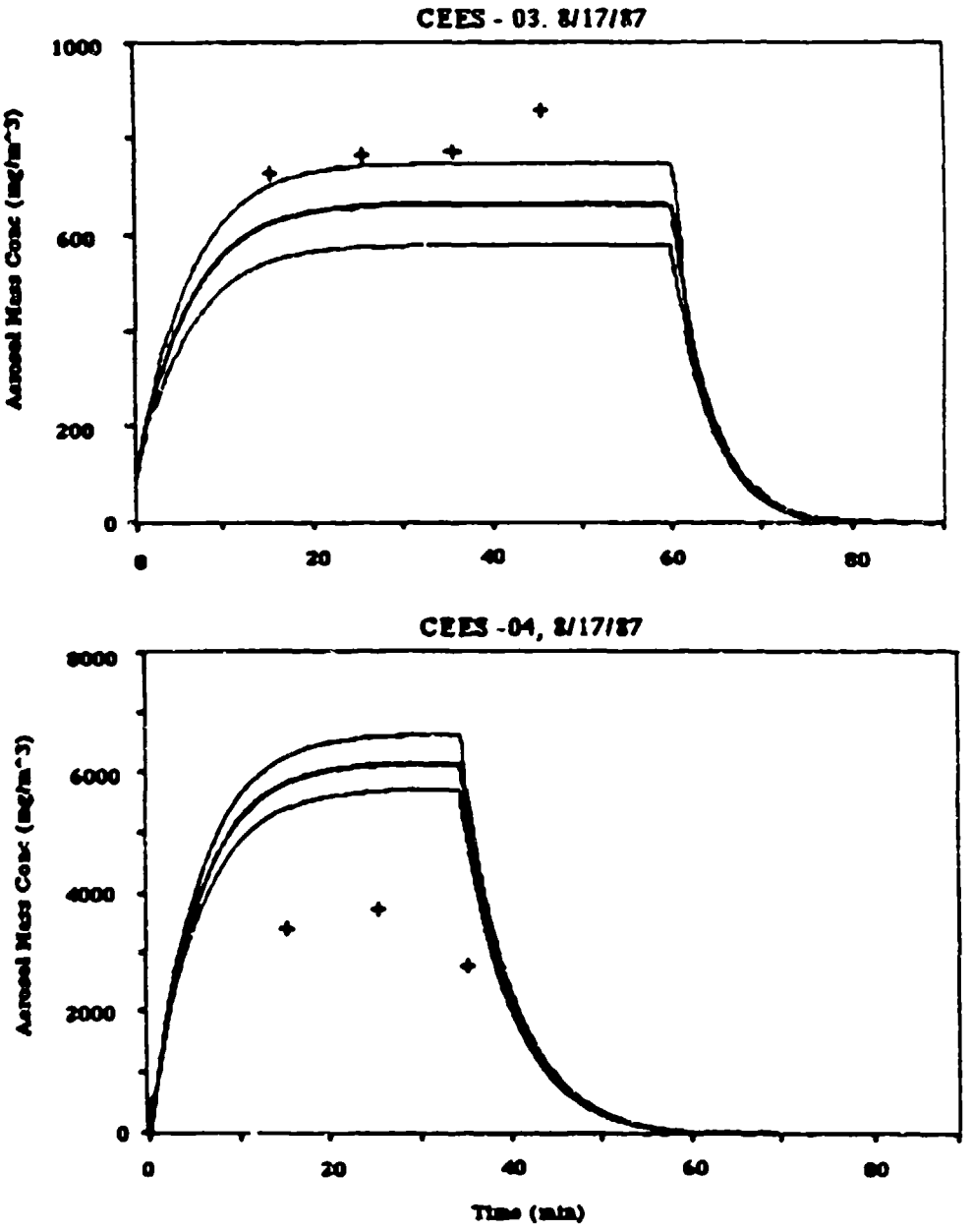


FIGURE 3.2. Predicted CEES Aerosol Concentrations Exiting the Exposure Region of the Henderson Chamber During Tests CEES-03 and CEES-04 (middle line). The lighter solid lines indicate the calculated uncertainties associated with all aspects of aerosol generation other than chamber losses. Data points on the plots represent CEES air concentrations determined from syringe grab samples.

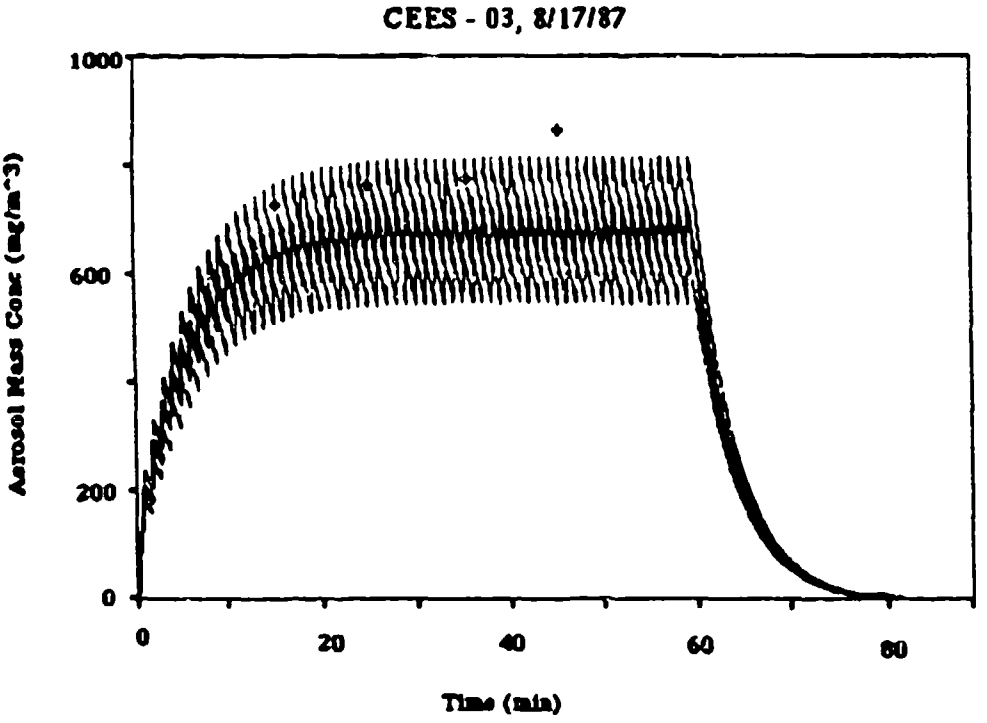


FIGURE 3.3. Predicted CEES Aerosol Concentrations Exiting the Exposure Region of the Henderson Chamber During Test CEES-03 (heavy center line). The lighter solid lines indicate the calculated uncertainties associated with all aspects of aerosol generation other than chamber losses. Data points on the plots represent CEES air concentrations determined from syringe grab samples.

TABLE 3.1. Predicted Aerosol Mass Concentration During CEES Tests

Test	Date	Test (mg/m^3)	Test + Purge (mg/m^3)
CEES-01	8/26/86	355 ± 45	255 ± 30
CEES-02	8/26/86	3940 ± 495	2850 ± 355
CEES-03	8/17/87	615 ± 75	440 ± 55
CEES-04	8/17/87	5240 ± 395	3340 ± 250

3.1.2 Bubbler Samples

Analysis of the bubbler samples indicated poor collection of CEES. This collection method was performed only for tests CEES-03 and -04. Airborne CEES concentrations were determined, summing measured levels of CEES, VES, and HEES. Levels of VES were rarely significant; levels of HEES were occasionally comparable to CEES. Levels of CEES found in the second bubbler were greater than those found in the first bubbler for both CEES tests. Therefore, no real estimates of sampling efficiency could be made. Other than relative measurement of CEES and its degradation products, the bubblers only provided an indication that the chamber concentration was greater than 5 or 10 mg/m^3. Because these values are approximately 100 times less than predicted concentrations, it was presumed that most of the sampled aerosol passed untrapped through the bubblers as vapor.

3.1.3 Syringe Samples

Limited data obtained during CEES-01 and -02 did not include a measure of the efficiency of the procedure of rinsing with water, although blanks were analyzed and found to have less than the analytical detection level. Results of samples obtained during CEES-02 may be ~10 times too low. It was not possible for the concentration during CEES-01 to be greater than during CEES-02 because of direct measure of the volumetric aerosol generation rate. Repeated gas washing of the syringe grab samples during CEES-03 and -04 indicated that little CEES remained in the syringe after the first hexane rinse. Analysis for CEES (CEES + HEES + VES) indicated that the efficiency of the first rinse was 93.2%. Table 3.2 shows aerosol mass concentration determined from analysis of syringe samples. Comparison of syringe and bubbler samples showed the aerosol mass concentration determined by bubbler samples to be ~0.005 times that determined by syringe samples.

3.1.4 Deposition Coupons

Analysis of deposits was completed by either HPLC or GC-MS. Samples contacted with water (CEES-01 and -02) were analyzed by HPLC-UV, and provided no detectable CEES, HEES, or VES. This may have been caused by an acid-catalyzed decomposition of CEES to an undetected product. It was found that the filters used to collect deposition materials would acidify 10 ml of pH 7.0 H_2O to a pH of 3.5. Samples contacted with hexane (CEES-03 and -04), and subsequently quantified using GC/MS revealed detectable quantities of CEES and HEES. Gravimetric analysis revealed limited information because the amount of mass collected was less than the detection limit of the analytical balance (±0.05 mg). It is thought that the high rate of evaporation of the CEES compounds contributed to the low mass determinations. A comparison of

TABLE 3.2. Aerosol Mass Concentration Determined by Analysis of Syringe Samples

Test	Sample Code	Time into Run (min)	Mass Concentration (mg/m^3)
CEES-01	AS-2	15	425
	AS-3	30	490
	AS-4	40	490
	AS-5	50	450
CEES-02	AS-2	15	210
	AS-3	30	160
	AS-4	40	170
	AS-5	50	185
CEES-03	AS-1	15	727
	AS-2	25	768
	AS-3	35	776
	AS-4	45	864
CEES-04	AS-1	15	3400
	AS-2	25	3750
	AS-3	35	2810
	AS-4	45	972

chemical and gravimetric analyses is shown in Table 3.3. Extremely low chemical results indicated that most of the CEES and its degradation products may have evaporated during the test and purge periods. Resultant deposition velocities, without consideration of probable significant evaporation, were ~10^{-5} cm/s for CEES-03, and ~10^{-6} cm/s for CEES-04.

3.1.5 Summary of Aerosol Concentration Results

Because concentration losses caused by deposition and adsorption cannot be predicted theoretically and because physical measurements showed conflicting results, actual aerosol mass concentration levels cannot be definitively reported. Extremely low results obtained from the bubbler samples were considered indicative of major sampling or analysis errors, and significant penetration of the bubbler traps was indicated; therefore the data were not considered reliable. Chemical analysis of syringe samples, on the other hand, generally provided consistent results, although the results for CEES-02 seemed to be ~10 times too low. Table 3.4 provides a comparison of the aerosol mass concentrations determined from averaged syringe samples with those calculated from known system characteristics. Syringe sample results were reduced by a factor equal to the ratio of the calculated test concentration to the test + purge concentration to provide a test + purge

TABLE 3.3. Mass Loading on Coupon During CEES Deposition Tests

Test	No. of Coupons	Gravimetric Analysis (mg)	Chemical Analysis (mg)	CEES/HEES/VES (%)
CEES-03	4	<0.01	0.00106 ± 0.00026	42.5 ± 4.1
CEES-04	6	<0.01	0.00095 ± 0.00067	44.7 ± 7.4

TABLE 3.4. Aerosol Mass Concentration Results for CEES Tests

Test	Target	Calculated		Measured		Measured/ Calculated
		Test	Test+Purge	Test	Test+Purge	
	(mg/m^3)					
CEES-01	100	355	255	465	335	1.31
CEES-02	1000	3940	2850	180	130	0.045
CEES-03	100	615	440	785	565	1.28
CEES-04	1000	5240	3340	3320	2120	0.64

concentration result. Both low-dose CEES tests show measured average aerosol concentrations greater than those calculated by a factor of 1.3. While the difference is minor, measured concentrations had been expected to be less than calculated concentrations as a result of system losses and chemical deposition.

Aerosol concentration during the high-dose tests indicated aerosol losses did occur in the exposure system. Sampling or analytical errors were the probable cause of very low measured aerosol concentrations during CEES-02. It is extremely unlikely that the mass of aerosol collected in syringe samples could have been less than that collected during CEES-01 (a low-dose test conducted on the same day) because of verified generation of ten times more CEES during the high-dose test than during the low-dose test. Results for CEES-04 indicate that the measured aerosol concentration was less than the calculated aerosol concentration by a factor of 0.64. This would be equivalent to a loss of aerosol on account of deposition and adsorption to chamber, plant

and soil surfaces equal to 0.17% to 0.25% of the aerosol per second. Only the first three CEES-04 syringe samples were considered because the fourth was obtained after cessation of aerosol generation, during the early purge period.

Possible errors in measurement and data interpretation include, but are probably not limited to the following:

- sampling losses at the inlet of the syringe needle
- diffusive and inertial losses at the bubbler inlet
- alterations in gas and droplet chemistry, which may have led to reduced levels of the primary chemicals as the aerosol, and deposits aged.

3.2 MASS LOADING ON AND DEPOSITION VELOCITIES TO FOLIAR SURFACES AND SOILS

A variety of physical and environmental factors combine to determine the rate at which atmospheric pollutants transfer from the air column, through the near-surface boundary layer, to a receptor surface. These factors, which include various atmospheric conditions such as wind speed and humidity, aerosol diameter, vapor pressure, and the physical and chemical structure of the receptor surface, combine to determine the net deposition to a surface. Two different removal mechanisms operate on atmospheric pollutants, one for gas-phase compounds and one for compounds found in the aerosol phase. Although the two deposition mechanisms depend on different parameters, the net deposition to a surface can be calculated when an average gas/aerosol deposition velocity is known. A deposition velocity, which is normally expressed as V_d in units of cm/s, is analogous to a mass transfer coefficient and describes the rate at which an atmospheric pollutant is deposited on a given surface. Without the deposition velocity, the dosing levels to a receptor surface cannot be predicted.

To quantify the relationship between chemical dosage and damage or effect, the aerosol concentration, deposition velocity, and surface exposure time must all be known to estimate the total surface deposition, or mass loading (ML). Because of the complexity of deposition processes, specific deposition velocities are rarely known, and therefore must be either measured directly or calculated. In this study we have directly measured surface ML by subsampling a known area of an exposed surface, and then extracting and quantifying any deposited chemical species. Subsequently, these data were combined with the measured aerosol concentrations and exposure times to compute the deposition velocity. The formula for calculating V_d is presented in Equation 1.

$$V_d\ (cm/s) = \frac{ML(ng\ compound/cm^2\ leaf)}{aerosol\ conc\ (ng\ compound/m^3)} \times \frac{1 \times 10^6\ cm^3}{m^3} \times \frac{1}{exposure\ time} \quad (1)$$

Quantifying the deposition process for specific receptor surfaces permits a comparison of the relative importance of atmospheric variables and canopy and receptor surface (plant and soil) characteristics to the net deposition efficiency. The following subsections discuss the ML of CEES to vegetative and soil surfaces, and the calculated deposition velocities are presented. All reported soil and plant results were obtained from exposure tests CEES-03 and CEES-04.

3.2.1 Vegetative Surfaces

The methodology used for plant exposure has been discussed in a previous section. Three species of plants were exposed during each test: two representatives of each species. Two tests were run, one at a low concentration and one at a high concentration. The results from the chemical analysis of the exposed plant tissues are given in Table 3.5.

The rapid decomposition of CEES in the presence of water necessitated that both the parent compound and the major decomposition products be quantified. The rate of CEES hydrolysis is exemplified by the ratio of HEES to CEES found on the receptor surfaces immediately after the exposure cycle was concluded. With the exception of the high-concentration exposure of the short needle pine, the decomposition product, HEES, was found at higher levels than the parent compound, CEES. Vinyl ethyl sulfide (VES) was not found above detection limits in any of the plant tissue samples. With tall fescue, a common prairie grass, the HEES concentration was two orders of magnitude greater than that of CEES at both high- and low-exposure concentrations. Results for tall sagebrush and short needle pine showed HEES concentrations to be only a factor of two higher than CEES. Based on the variations of the HEES/CEES ratio found on the different plant species and the relative lack of significant quantities of HEES in the aerosol, it appears that the hydrolysis took place on the receptor surface. Differences in the ratio of HEES to CEES on different plant surfaces may reflect the relative amounts of free water on or in their leaf tissues.

It should be noted that during the high-dose experiment, the average concentration of CEES and decomposition products in the exposure air were roughly 3.5 times greater than in the low-dose test, as determined by analysis of syringe samples. In spite of this, the ML to the plant surfaces only increased by an average factor of 1.7. Some of this difference is probably caused by the high vapor-pressure of CEES. Much of the generated aerosol appears to have rapidly evaporated, in which case the net ML to the plant surface would be the

TABLE 3.5. Mass Loading of CEES, HEES, and VES on Plant Surfaces[a]

Plant Species/ Exposure Test	Chemical Species			
	VES	CEES	HEES	HEES/CEES
TALL FESCUE				
Low Conc	<1	1.2 (0.2)	420 (70)	350
High Conc	<1	4.0 (2)	700 (200)	175
SAGEBRUSH				
Low Conc	<1	240 (10)	520 (50)	2.1
High Conc	<1	670 (20)	1100 (300)	1.6
SHORT NEEDLE PINE				
Low Conc	<1	190 (30)	400 (30)	2.1
High Conc	<1	360 (33)	270 (4)	0.8

(a) Concentration in ng/cm^2 leaf area. Values are given as average (error of the mean). Unless otherwise noted, n=2. Detection limit was 1 ng/cm^2 leaf surface.

sum of aerosol deposition to, and gas adsorption by, the plant surfaces, minus any evaporative losses from surfaces.

The parameters which influence gas adsorption to a surface are highly dependent on the chemical composition of the gas and how that compound absorbs to, or interacts with the adsorption sites on the surface. A gas can also dissolve into the leaf cuticle, partitioning between the gaseous and dissolved states on the basis of Henry's Law constant. Chemically, this is a more complex situation than direct aerosol deposition, since aerosol deposition is generally dependent on the aerodynamic mass median diameter of the particles and relatively independent of an aerosol's chemical composition. The data indicate that for CEES there may be a limited number of adsorption sites on the plant receptor surfaces and that they are reaching a point of saturation during the high-dose experiments.

3.2.2 Soil Surfaces

The results from the soil exposure tests are presented in Table 3.6. The CEES appears to have been rapidly converted to HEES, just as it was on the plant surfaces. Again, VES was not found in quantities above the detection limit. There was a significant difference between the MLs on soil from the low-dose

TABLE 3.6. Mass Loading of VES, CEES, and HEES on Soil Surfaces[a]

Soil/ Exposure Test	Chemical Species			
	VES	CEES	HEES	HEES/CEES
MAXEY FLATS				
Low Conc	<10	<10	410 (6)	>41
High Conc	<10	70 (30)	2200 (70)	31
BURBANK				
Low Conc	<10	33 (10)	1300 (700)	39
High Conc	23 (7)	220 (14)	5900 (200)	27

(a) Mass loading given in ng/cm^2 soil surface. Values are given as average (s.d., n=4). Detection limit was 10 ng/cm^2 soil surface.

low-dose and high-dose experiments compared MLs foliar surfaces. The high-dose loadings were about five times greater than the low-dose loadings and similar to measured aerosol concentrations. This implies that gaseous adsorption was playing an important role in transferring the material to the soil surface, with substantially less revolatilization than was noted for foliar surfaces. Thus, the soil surfaces appear to provide a greater opportunity for gas adsorption than the plant surfaces. The Burbank soil showed an ML three times greater than the Maxey Flats soil. Clearly, this is a function of differences in either the number or type of adsorption sites found on the two soils. What is not clear is if the adsorption is taking place on organic matter or at cation-exchange sites (Table 2.3). The ratio of HEES to CEES recovered from these soils is similar overall at 27 to 41. A more in-depth study would be necessary to precisely define the mechanism of CEES/HEES adsorption on these soils.

3.2.3 Aerosol Deposition Velocities for Plants and Soils

Net deposition velocities (Table 3.7) that were computed using the MLs, exposure times, and measured air concentrations are given in Tables 3.4 and 3.6. These values are termed "net" because the high vapor-pressure of CEES resulted in evaporation of an unknown portion of the generated aerosol before, and possibly after, deposition on the receptor surface. Therefore, these values represent the average V_d from both aerosol and gas-phase deposition.

TABLE 3.7. Net Deposition Velocity of CEES to Plant and Soil Surfaces

Plant/ Soil Surface	Deposition Velocity[a], cm/s x 10^3 (± s.d.) Low-Dose	High-Dose
Tall Fescue	0.15 (0.02)	0.11 (0.04)
Sagebrush	0.27 (0.01)	0.27 (0.04)
Short Needle Pine	0.21 (0.02)	0.090 (0.006)
Maxey Flats Soil	0.15 (0.01)	0.32 (0.008)
Burbank Soil	0.21 (0.1)	0.87 (0.004)

[a] Mean ± error of the mean, n=2.

In general, the net deposition velocities to the plant surfaces were relatively similar, with an average value of 0.18 x 10^{-3} (±0.08) cm/s. This can be compared to the net deposition velocities measured in previous experiments using diisopropyl fluorophosphate (DFP, V_d 0.42 x 10^{-3}), another simulant with a relatively high vapor pressure. Although the computed V_d for CEES to plant surfaces is in this range, it is about 50% lc This difference is probably due to the increased volatility of CEES, which r in loss aerosol deposition and increased evaporation of deposited CEES.

The V_d values for soils showed that there was a significant difference between the two soil types ($P \geq 0.01$), as well as between the two dosing concentrations. Both soil types showed increased deposition velocities during the high-dose experiments, with values of V_d calculated at 0.15 and 0.32 x 10^{-3} cm/s for Maxey Flats, and 0.21 and 0.87 x 10^3 cm/s for Burbank soil, for the low and high doses, respectively. The higher V_d values for Burbank compared with Maxey Flats again suggests that gas-phase adsorption played a significant role in the deposition mechanism transferring CEES to the surface soils. If sedimentation processes dominated as would be expected for aerosols, V_d values should be similar between dose treatments and for both soils.

3.3 PHYTOTOXICITY

3.3.1 Gross Phytotoxicity

The determination of foliar toxicity effects can present several problems. While the plants used for this study were of natural genetic stock, individual differences in physiological variability and toxicity response within each test species could be expected to occur. Further, under both field conditions and in the stirred exposure system employed for these studies, where air movement occurs along a given vector (i.e., wind direction), substantial amounts of deposition to canopies can occur irregularly depending on canopy structure and density and the presence of back eddies. Finally, the most cost-effective and consistent manner in which to quantify damage to vegetation must be considered. Taking these issues into account, a nonparametric grading system, a modified Daubenmire rating scale (MDRS) (Daubenmire 1959), can be used as a damage index for the evaluation of contact toxicity.

The criteria used to compile the MDRS are described in Table 3.8. These criteria were used to describe the extent of visual damage to the plants caused by the given exposure period at each concentration of CEES (Tables 2.1 and 2.2). Damage can be identified by any one of the listed symptoms; however, effects appeared to be limited to tip burn, necrotic spotting, leaf curl, chlorosis, leaf drop, and death as given in Table 3.9. The intensity of foliar damage was further quantified by determining the physical length of the necrosis or leaf damage. The data generated are nonparametric and represent an estimate of foliar damage.

In both exposure series, the short needle pines proved the most sensitive to the CEES as compared to the other two species (Table 3.9). Within 72 h, almost half of the needles on all three exposed plants in the low-concentration experiment exhibited chlorosis and tip burn. These symptoms spread to all the foliage of the pines after another 5 days. All three exposed to high concentrations developed chlorotic symptoms within 24 h, with two of the three plants dying within a week and the third dying after 2 weeks. In the plants exposed to low concentrations new buds emerged within 3 weeks after exposure and did not appear to be affected indicating that the phytotoxic effects may have been of a contact nature and not transported within the plant to the younger tissues.

The sagebrush did not develop visual phytotoxic symptoms until 7 days after exposure for both the low- and high-concentration tests (Table 3.9). The most severe effects were the leaf drop that occurred on all of the plants in both concentrations. The sagebrush had not begun to recover from the exposure and no new growth was observed after 3 weeks, when the plants were terminated.

TABLE 3.8. Coding for Modified Daubenmire Rating Scale and Associated Phytotoxicity Symptoms

Symptom/Intensity	Description
Modified Daubenmire Rating Scale	
0	no obvious effects over controls
1	5% of plant foliage affected
2	between 5%-25% of foliage affected
3	between 25%-50% of foliage affected
4	between 50%-75% of foliage affected
5	between 75%-95% of foliage affected
6	between 95%-100% of foliage affected
Phenotypic Responses	
OGA	old growth affected
NGA	new growth affected
O&NGA	old and new growth affected
TB	tip or leaf edge burn
LBD	leaf burn and leaf drop
NS	necrotic spotting
LD	leaf abscission or needle drop
Chl	chlorosis
BD	blade dieback
LC	leaf curl
W	wilting
GD	growing tip dieback
D	plant dead
F/SA	floral or seed/fruit abortion
(value)	indicates the length in cm that needles or leaves exhibit dieback or burn

TABLE 3.9. Phenotypic Responses of Several Plant Species Following Foliar Exposure to CEES

Treatment/ Plant Species	Days of Post-Exposure		
	1	3	8
	Toxicity Rating		
Low-Dose			
Short Needle Pine	0	3,chl,TB	5,TB,NS
Sagebrush	0	0	4,TB,LC,LD
Tall Fescue	0	0	3,chl,LC
High-Dose			
Short Needle Pine	3,chl	5,NS,LBD	D
Sagebrush	0	0	6,LC,LD
Tall Fescue	0	0	4,chl,TB,LD

Visually, the tall fescue exhibited the least damage of the three exposed species. After a week, almost half of the leaves on the plants exposed to low concentrations and about three-fourths of those exposed to high concentrations developed chlorotic spotting. Further, all of the plants exposed to high concentrations showed some additional tip burn and leaf drop in their canopies (Table 3.9). Younger tissues and newly emerging shoots, however, were normal in appearance and grew at rates comparable to controls. This indicated that there were no lasting effects of the CEES on the grass. Furthermore, when pots of soil were exposed and then seeded with the grass, germination and growth in all four pots proceeded at the same rate as in control pots, even after 3 months.

3.3.2 Metabolic Effects on Plants

Whole Plant Measurements

Severe phytotoxic effects were not observed for 3 to 8 days after foliar exposure to CEES. In all species tested, these effects were limited to contacted tissues while the new growth appeared healthy. This would suggest that the CEES, although absorbed into the tissues of the leaves, was not mobile and/or persistent within the plant. However, even though there was a delay in the onset of visible symptoms, their appearance was an indication that adverse metabolic effects did precede them. An early indication of damage may be obtained through assays of basic plant metabolic processes, such as photosynthesis and respiration. The activity of these reactions may be followed through either the uptake and evolution of oxygen by respiration and photosynthesis or the uptake and evolution of carbon dioxide by photosynthesis and respiration.

Oxygen exchange may be conveniently followed polarigraphically using intact leaf segments. Samples were taken from leaves of all three species before and following exposure to CEES, for both the high- and low-concentration dose runs. The results of these studies are given in Figures 3.4 to 3.6. Oxygen evolutions which is indicative of relative changes in photosynthesis or growth, is expressed as a positive value, while respiration (oxygen uptake) is expressed as a negative function. All data points are the averages of three paired runs (6 samples) and are given with error bars equal to the standard deviations.

Within 24 hours after exposure in the low-concentration experiments, the sagebrush responded with a significant elevation in photosynthetic rates and apparent increases in dark respiration (Figure 3.4). Similar photosynthetic responses as well as significant increases in dark respiration were observed in the sagebrush plants exposed to high concentrations (Figure 3.4). However, within 48 h, rates of photosynthesis and respiration returned to pre-exposure levels in plants from both treatments. Following this period, photosynthetic and respiratory levels for the low-concentration plants remained fairly

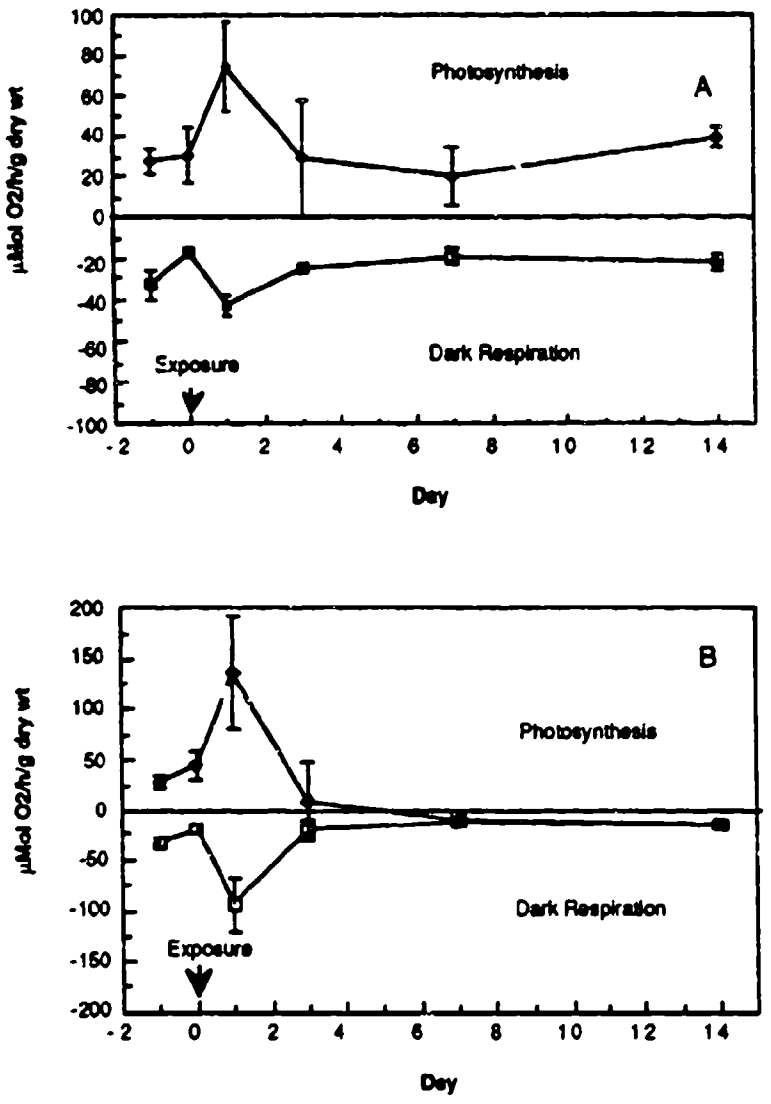


FIGURE 3.4. Photosynthesis (O_2 evolution) and Dark Respiration (O_2 uptake) in µMol O_2/h/g dry wt of Isolated Leaf Segments from Sagebrush Before and After Exposure to CEES. Data points are averages of six assays with error bars equal to ± s.d.; A and B designate low- and high-concentration exposures, respectively.

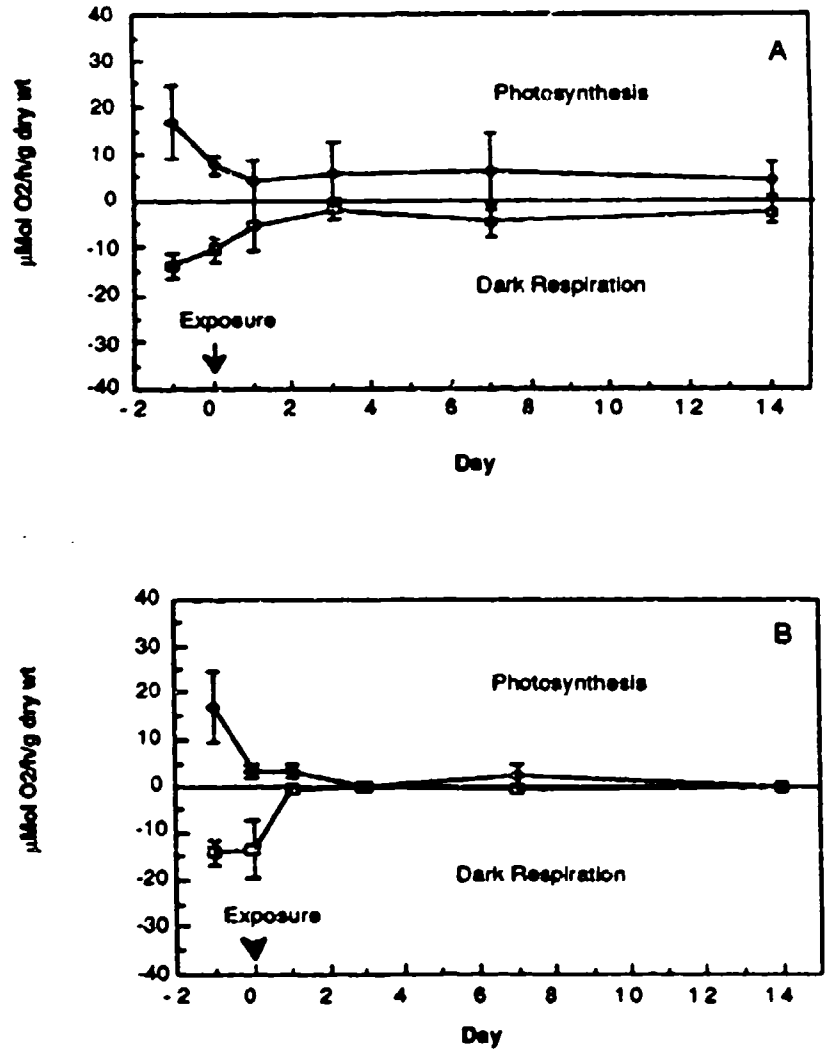


FIGURE 3.5. Photosynthesis (O_2 evolution) and Dark Respiration (O_2 uptake) in µMol O_2/h/g dry wt of Isolated Leaf Segments from Short Needle Pine Before and After Exposure to CEES. Data points are averages of six assays with error bars equal to ± s.d.; A and B designate low- and high-concentration exposures, respectively.

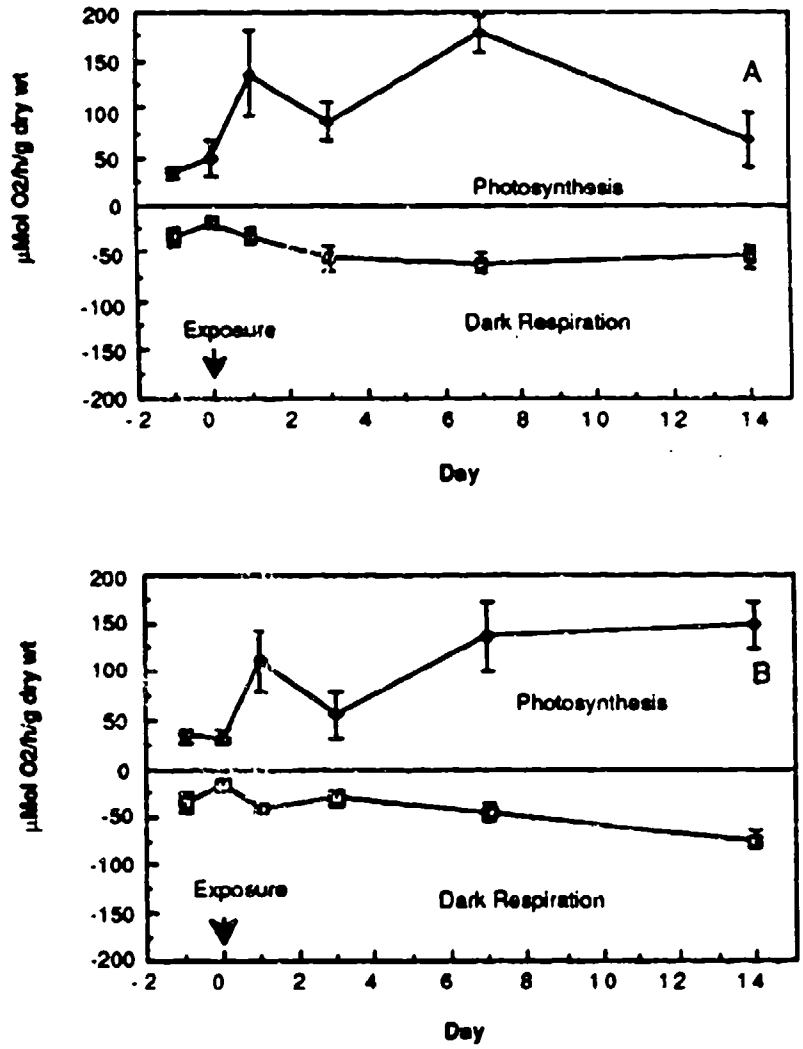


FIGURE 3.6. Photosynthesis (O_2 evolution) and Dark Respiration (O_2 uptake) in µMol O_2/h/g dry wt of Isolated Leaf Segments from Tall Fescue Before and After Exposure to CEES. Data points are averages of six assays with error bars equal to ± s.d.; A and B designate low- and high- concentration exposures, respectively.

constant over the next 2 weeks, with a slight elevation in photosynthesis after 14 days (Figure 3.4). This may indicate that the tissues remaining on the plants, even though exhibiting some phytotoxic symptoms (Table 3.9), were physiologically recovering from the chemical insult. The plants exposed to high concentrations, however, did not recover, and their photosynthetic ability was apparently lost after 7 days. Their respiration increased only slightly during this period possibly indicating severe metabolic damage from the exposure. It would be difficult to predict a recovery for these plants over a long period of time.

The short needle pine exhibited the most dramatic response to the simulant of the three plant species (Figure 3.5). Immediately following exposure at both concentrations, there were declines in both photosynthesis and dark respiration. In the plants exposed to low concentrations, some photosynthetic ability was evident over the next 2 weeks, but it averaged less than that of the control. However, there was also large variability in these samples that was dependent upon canopy location (Figure 3.5A). Similar trends were evident in the dark respiration rates. Plants exposed to the high concentration lost all photosynthetic and respiratory capacity within 72 h following exposure (Figure 3.5B). Observed phytotoxic damage, although extensive, did not reveal the totality of the apparent damage at this early stage.

The plant species apparently least affected by the simulant was the grass, although responses similar to those observed in the other plant species did occur. In plants exposed to either the high or low concentration, an elevation in the rate of oxygen evolution was observed within 24 h (Figure 3.6). This level of photosynthesis was not maintained, and the rates declined somewhat after 72 h. Plants exposed to either high or low concentrations of CEES continued to fluctuate in their photosynthetic rates over the next 2 weeks. However, growth was maintained at a level equal to that of the controls. Again, these variations in photosynthesis may have been due to sampling, given the basepetal growth pattern of the monocots. Respiration rates for plants exposed to either dose concentration were depressed slightly following exposure but then remained constant in the low-exposure plants and increased in the high-exposure plants over the next 14 days (Figure 3.6).

These results indicate that metabolic events were occurring within the leaves prior to the onset of phytotoxic symptoms. However, there also appeared to be a slight delay in the onset of these effects, most likely caused by the time required for the simulant to penetrate the leaves. Further, the elevation in oxygen evolution observed after 24 h in all species indicated that some reaction could be occurring within the chloroplasts of the leaves, specifically in the photosynthetic electron transport system involving the splitting of water and concomitant production of oxygen. Since whole-leaf measurements would not

yield further information to resolve this question, it was felt that additional studies were required.

Isolated Chloroplasts

There are few reproducible techniques available in the literature for producing isolated chloroplasts from any of the species exposed in the wind tunnel experiments. It was therefore decided that a representative plant species, namely spinach (*Spinacea oleraceae*), would be useful, since procedures do exist for the routine isolation of high-quality chloroplasts from its leaves. Furthermore, the use of the isolated organelles would provide information on the direct effects of CEES on photosynthesis without having to first traverse the cell membrane, and assays could be run on control and treated organelles at the same time.

The electron transport chain of the light-reaction photosystems consists of two separate photoacts, or photosystem reaction centers (PS I and PS II), with accompanying light-harvesting pigment/protein complexes. Both are located on the interior of the thylakoid membrane (although PS I may be closer to the outer, or stromal side). The water-splitting site is located on the inner thylakoid membrane, while the other end of the redox potential gradient, the site of $NADH_2$ production, is located on the outer stromal surface. A number of intermediate electorn carriers span the membrane and aid in the transfer of protons to the interior of the thylakoid. To determine the probable site of action by the CEES, measurements were taken from both photosystems as well as the intact chain. The results, expressed as percent of measurements obtained from controls in paired experiments, are given in Table 3.10.

At low concentration (1 ppm), CEES does not appear to have any significant effect on whole-chain activity, although there is a slight depression (Table 3.10). This is also true of the PS I measurements, which are similarly depressed but not significantly so. There is, however, a significant elevation in the activity of PS II following exposure. This is the site of oxygen evolution and would account for the similar rise seen in the whole leaves.

At a higher concentration (10 ppm), severe inhibition of the electron transport chain occurs over the whole chain, as well as in the PS I portion (Table 3.10). There is apparently no significant inhibition at PS II although the average was slightly higher than the controls. This lack of significant inhibition, compared to the 1 ppm treatment, may be due to more severe damage from the higher CEES concentration resulting from increased penetration of CEES to the inner side of the thylakoid membrane.

TABLE 3.10. Interaction of CEES with Electron Transport Systems of Isolated Spinach Chloroplasts[a]

CEES Concentration (ppm)	Electron Transport System (O_2 Evolution), % Control ± s.d.		
	Whole Chain	Photosystem I	Photosystem II
b			
1	94.19 ± 3.0[b]	86.61 ± 25.7[b]	131.75 ± 1.59[c]
10	26.99 ± 3.99[c]	29.54 ± 1.85[c]	116.99 ± 15.32[b]

[a] *In vitro* amendment of simulant; exposure duration approximately 1 h. Data are avg ± s.d., n=3.
[b] Not significant based on two-tailed t-Test.
[c] Level of significance based on two-tailed t-Test $P < 0.01$.

In the electron transport chain of chloroplasts, PS II precedes PS I and is itself preceded or paralleled by the water-splitting site. If a disruption in the chain were to occur between PS II and PS I or at the outer thylakoid membrane portion of the PS I segment, there would be a depression of the activity of the whole chain as well as PS I. An uncoupling of this portion from the interior PS II and water-splitting segments might account for the rapid acceleration of oxygen evolution. Continued loss of the PS I and associated $NADH_2$ production activity would result in a loss of photosynthetic carbon assimilation capability, as well as other metabolic processes dependent on strong reductants within the chloroplasts, such as photorespiration and transamination reactions. These losses may eventually prove fatal to the organism.

The maintenance of PS II activity indicates that the CEES does not apparently penetrate the thylakoid membrane quickly but may act upon the surface proteins, particularly those containing sulfhydryls. Prolonged exposure, not followed in these isolated organelle experiments, may show a further loss of PS II activity as the compound penetrates the membrane.

3.4 EFFECTS ON SOIL MICROBES

The effect of CEES on soil microbial activity was studied using Burbank and Palouse soils. Maxey Flats soil was not studied because of its general infertility and low microbial activity. The inhibition of enzymes that drive key metabolic reactions in microbial cells is likely the underlying cause of toxicity of chemicals to soil microorganisms. Microbial dehydrogenase enzyme systems

catalyze the oxidation of organic material and fulfill an important role in the soil/carbon cycle. The assay of soil dehydrogenase activity is a general indicator of the potential activity of the soil microbial population (Skujins 1976). Phosphatases, which can exist extracellularly, are a broad group of enzymes that cleave esters and anhydrides of phosphates from complex organophosphates and are believed to be important in the mineralization of this element from organic matter in soil (Ramirez-Martinez 1968). Thus, these two enzymatic activities are used in this study to assess CEES toxicity toward microorganisms and biochemical processes in soil.

The extent of inhibition produced by CEES of enzymatic activities in soil is found to be dependent on soil type, CEES concentration, and length of incubation. The relationship between soil dehydrogenase in soil activity and CEES concentration at three incubation periods can be described by the equations derived from power curve fitting as shown in Figure 3.7. Immediately after the addition of CEES (after an incubation time less than 30 min), dehydrogenase activity in Burbank soil was not significantly affected by concentrations of up to 250 µg/g (Table 3.11). However, after one week's incubation time, the activity dropped to about 60% at the highest concentration of CEES tested. In Palouse soil, CEES exerted a more instantaneous effect on dehydrogenase activity in soil. Initial inhibition was acute and persisted for at least 4 weeks at the higher concentration of CEES (250 µg/g). However, dehydrogenase activity recovered after 4 weeks in Palouse soil treated with the lower concentration of CEES (5 to 10 µg/g).

The relationship between CEES concentration and its effect on soil phosphatase activity is shown in Table 3.12, with the microbial inhibitions being represented as a power curve functions. as power curve fit equations. In Burbank soil, phosphatase activity in soil amended with a low concentration of CEES (5 to 10 µg/g) increased slightly when measured immediately after the addition of CEES and also after incubation for 1 week (Figure 3.8). At the same time, about 12% to 30% of the activity was inhibited by concentrations of CEES greater than 10 µg/g. The inhibition increased with incubation time. After 4 weeks of incubation, phosphatase in Burbank soil decreased by about 40%. In Palouse soil, the initial effect of low concentrations of CEES on phosphatase activity were negligible. However, the activity decreased 20% at concentrations greater than 10 µg/g. After incubation for 4 weeks, the phosphatase activity decreased by about 30% in Palouse soil.

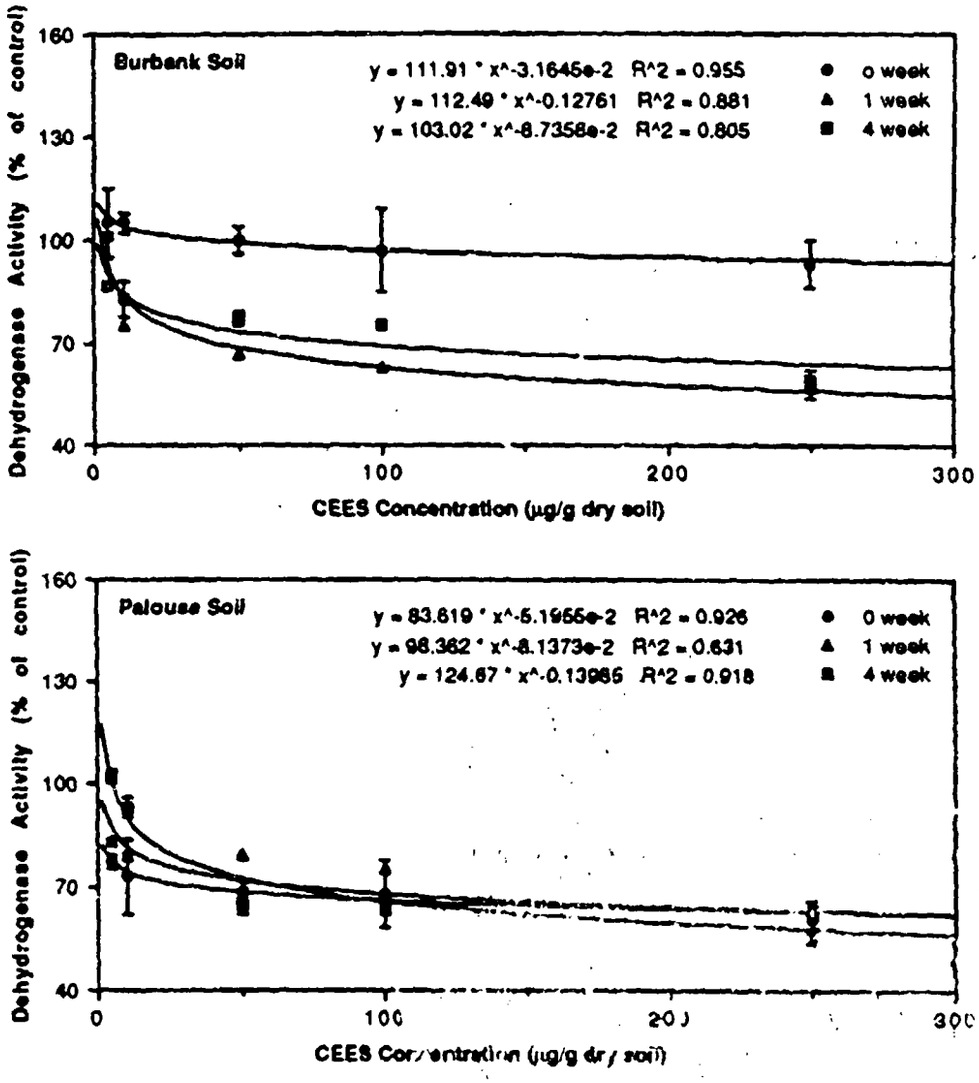


FIGURE 3.7. Influence of CEES Soil Concentrations on Dehydrogenase Activity in Burbank and Palouse Soils. (Error bars represent s.d., n = 2.)

TABLE 3.11. Effect of CEES on Dehydrogenase Activities in Burbank and Palouse Soils

CEES Conc. (µg/g)	Dehydrogenase Activities (% of Control[a])					
	Burbank Soil Incubation (weeks)			Palouse Soil Incubation (weeks)		
	0	1	4	0	1	4
0	100 (4)	100 (1)	100 (1)	100 (3)	100 (0.5)	100 (3)
5	105 (10)	101 (1)	87 (1) *	77 (2) *	83 (1) *	102 (2)
10	105 (3)	75 (0.3)*[b]	83 (5) *	73 (11) *	79 (1) *	93 (3)
50	100 (4)	67 (0.2) *	77 (2) *	69 (2) *	79 (0.4) *	65 (3)*
100	97 (12)	63 (0.1) *	75 (1) *	68 (10) *	75 (0.3) *	64 (2)*
250	93 (7)	57 (3) *	58 (4) *	61 (4) *	55 (6) *	62 (4)*

(a) mean (± s.d.), n=2.

(b) * denotes significant difference from control based on Student's t-test, P≤0.05.

TABLE 3.12. The Effect of CEES on Phosphatase Activities in Burbank and Palouse Soils

CEES Conc. (µg/g)	Phosphatase Activities (% of Control[a])					
	Burbank Soil Incubation (weeks)			Palouse Soil Incubation (weeks)		
	0	1	4	0	1	4
0	100 (9)	100 (0.2)	100 (2)	100 (11)	100 (8)	100 (7)
5	113 (8)	105 (8)	80 (1)*	97 (8)	83 (15)	94 (4)
10	108 (10)	96 (6)	75 (2)*	90 (9)	78 (8)	82 (4)*
50	88 (9)	75 (0.3)*[b]	74 (1)*	83 (7)	75 (13)	81 (6)*
100	88 (6)	74 (0.4)*	67 (1)*	80 (6)	72 (5)*	68 (4)*
250	88 (7)	69 (5)*	62 (1)*	79 (6)	70 (5)*	68 (6)*

(a) mean(± s.d.), n=2.

(b) * denotes significant difference from control based on Student's t-test, P≤0.05.

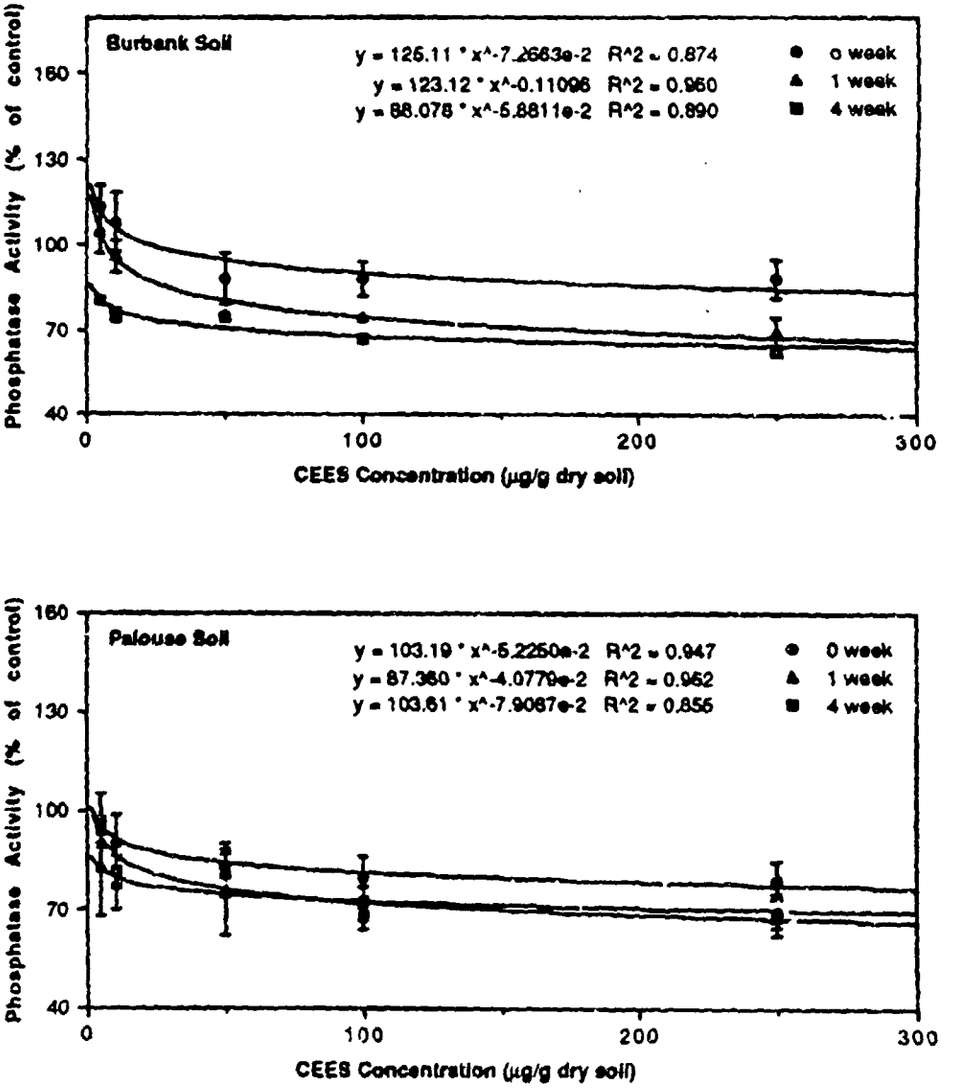


FIGURE 3.8. Influence of CEES Soil Concentrations on Phosphatase Activity in Burbank and Palouse Soils. (Error bars represent s.d., n = 2.)

The data show that low concentrations of CEES (5 to 10 µg/g dry soil) do not significantly impact soil enzymatic activities. However, at concentrations greater than 10 µg/g, activities can be seriously impacted. These data indicate that CEES may be metabolized at low concentration. The aliphatic structure of CEES suggests this compound may be degraded by soil concentrations but becomes toxic at higher concentrations. Although enzyme activities decreased with incubation time, the most severe inhibition did not exceed 50% with the highest dose (250 µg/g) tested.

3.5 TERRESTRIAL PERSISTENCE

In the following sections, the results of the CEES tests are discussed in terms of persistence of CEES in soil and on foliar surfaces.

3.5.1 Persistence in Soil

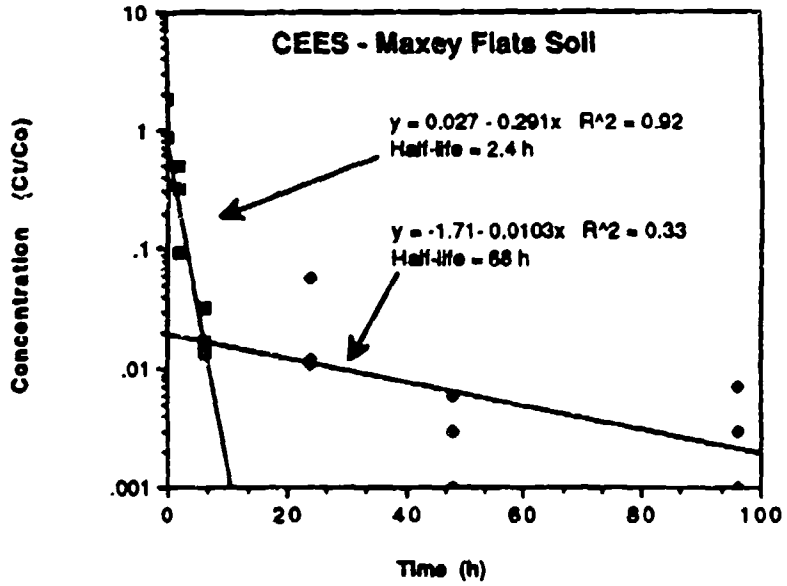
The persistence of CEES and its decomposition products, HEES and VES, was evaluated following airborne deposition of the compounds to soil surfaces. In the case of both Maxey Flats and Burbank soils, the fraction of CEES remaining on soil surfaces rapidly decreased over the 1- to 4-h period following deposition (Figures 3.9 and 3.10). A rapid decline in CEES occurred in the first 0.9- and 2.4-h period following contamination of Burbank and Maxey Flats soil, respectively. The initial half-life of CEES with 0.9 and 2.4 h for Burbank and Maxey Flats soil, respectively. This was followed by a slower decline to detection limits after 96 h. The overall half-life for the second depuration isotherm was 59 and 68 h for these two soils.

The concentrations of HEES in Maxey Flats and, particularly, Burbank soil tended to increase over the 96-h period of analysis. This would suggest that HEES was substantially more persistent than CEES. There were no indications that VES has formed in detectable quantities on short-term incubation in soils.

3.5.2 Persistence on Foliar Surfaces

Plant foliage was contaminated with aerosolized CEES and the foliar tissues extracted and analyzed for CEES, HEES, and VES for 30 h following exposure. The depuration of CEES and HEES deposited to foliage of sagebrush, short needle pine and tall fescue are shown in Figures 3.11, 3.12, and 3.13, respectively. As with soils, no VES was found to be present in extractable quantities.

The major extractable species associated with all foliage types was HEES. While CEES was present and detected during early sampling periods, its concentration was substantially less than that of HEES, and its depuration rate



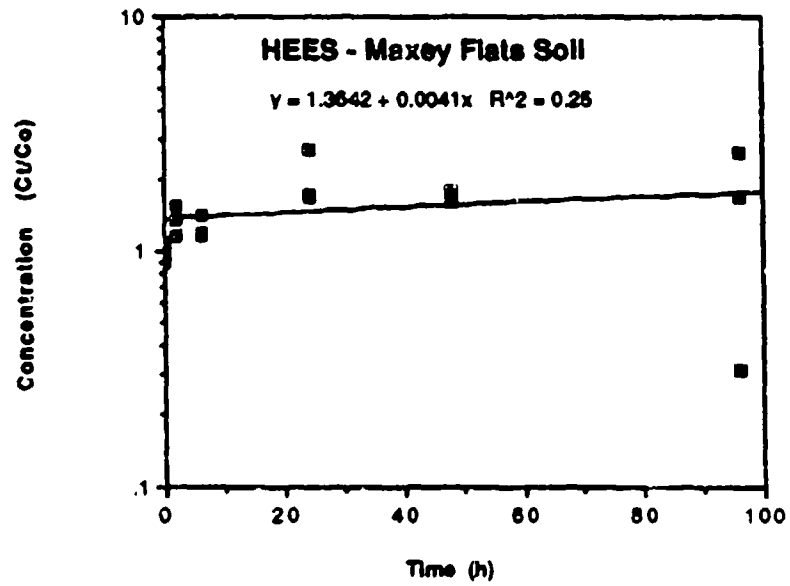
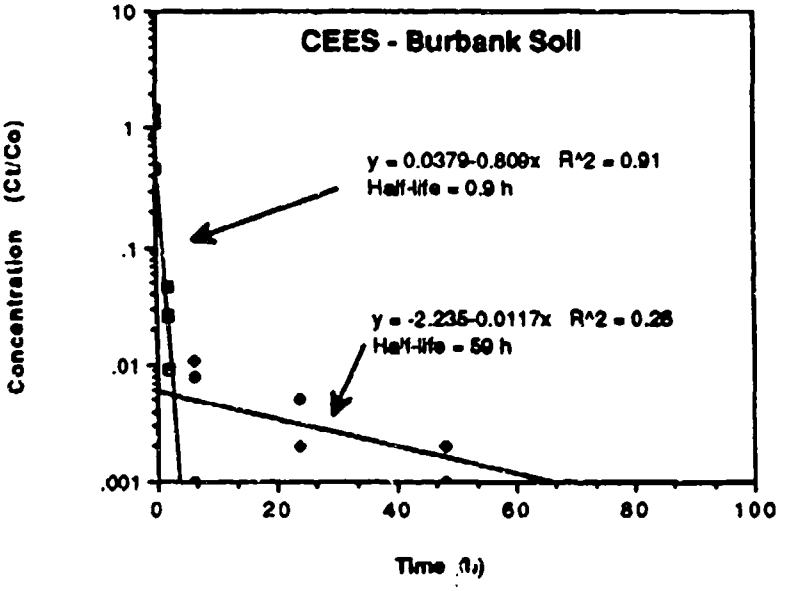


FIGURE 3.9. Persistence of CEES and HEES in Maxey Flats Soil



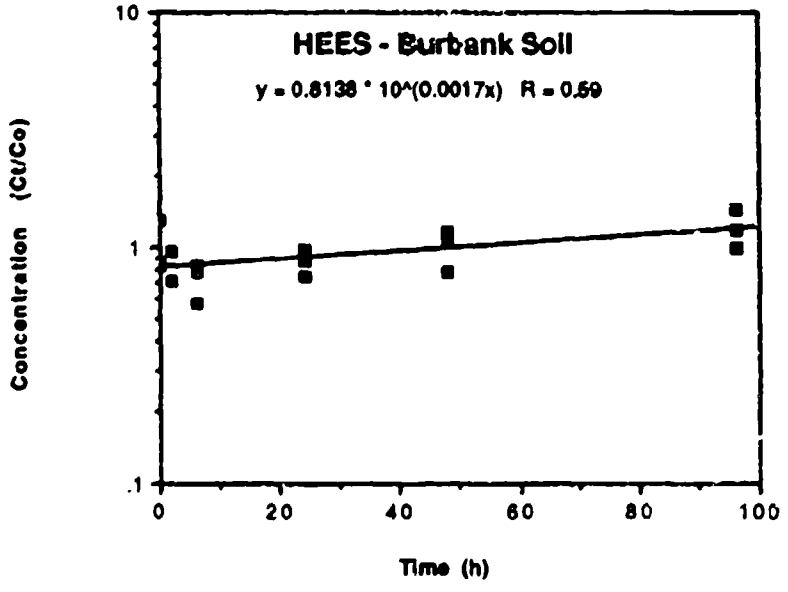


FIGURE 3.10. Persistence of CEES and HEES in Burbank Soil

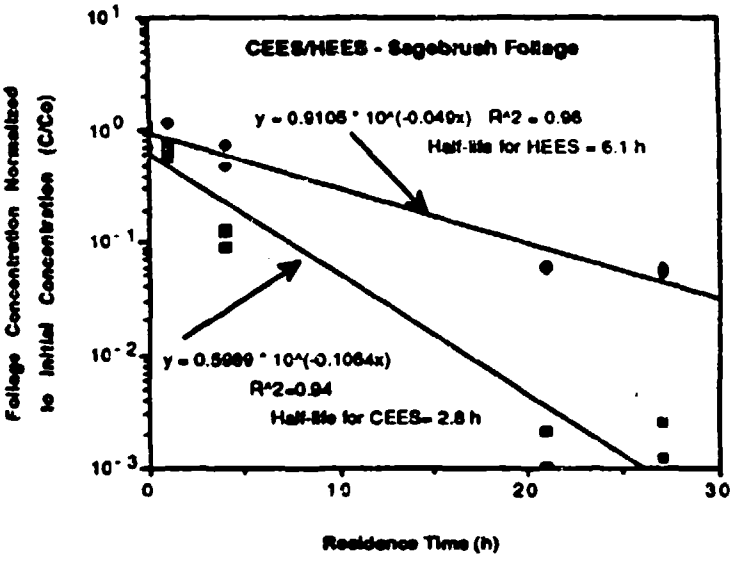


FIGURE 3.11. Residence Time of CEES and HEES on Foliar Surfaces of Sagebrush

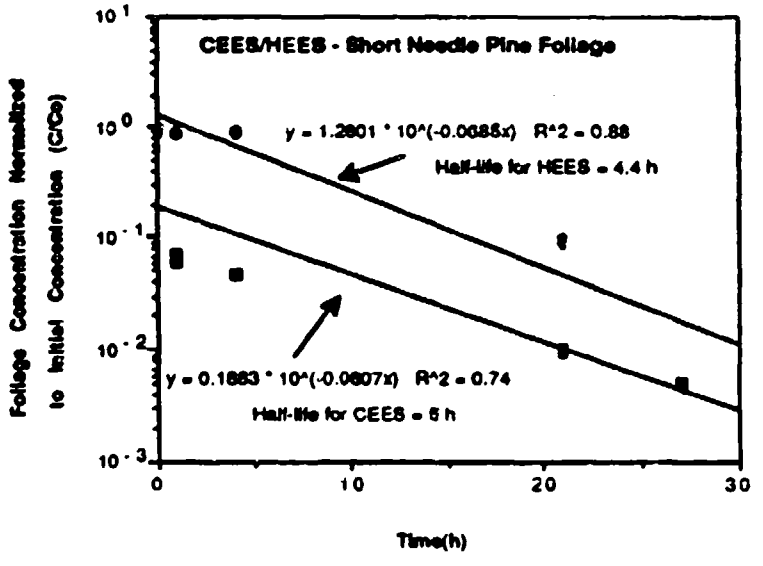


FIGURE 3.12. Residence Time of CEES and HEES on Foliar Surfaces of Short Needle Pine

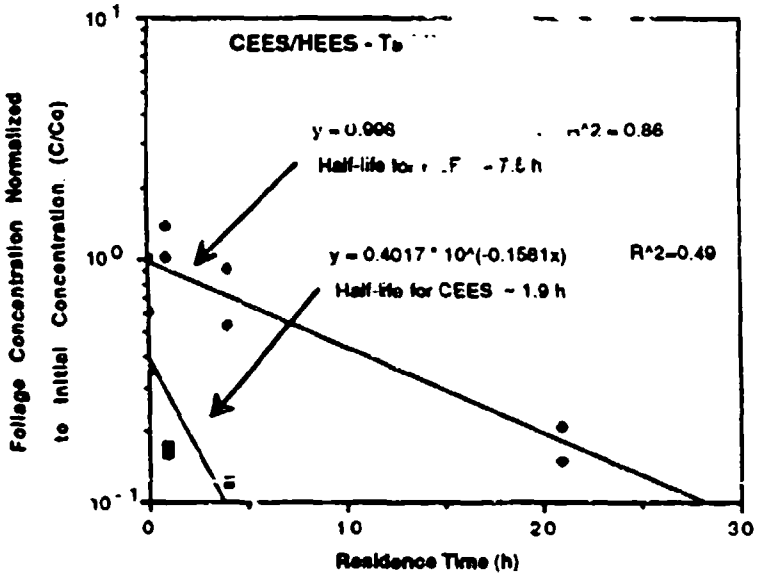


FIGURE 3.13. Residence Time of CEES and HEES on Foliar Surfaces of Tall Fescue

was greater than HEES for two of the three plant species. The half-life of CEES deposited to foliar surfaces ranged from 2 to 5 h, while that of HEES ranged from 4 to 8 h.

3.6 AQUATIC BEHAVIOR

The chemical behavior and biological effects of CEES were examined in laboratory tanks containing lake water. Existing information suggested that CEES has relatively low water solubility (1.7 mg/ml) and, therefore, may concentrate on the water surface. No information was available on the kinetics of movement of CEES into the water column following application or deposition to the water surface. Although it is believed to hydrolyze in water to form HEES and possibly other compounds (Sadowski et al. 1983; Bossle et al. 1984), information on its chemical stability in water over time was not available. Assessments of the toxicity of CEES have been limited to a few laboratory animal (rat, mouse, and rabbit) studies. No information was available on its toxicity to aquatic organisms.

The objectives of our study were to 1) determine the behavior and stability of CEES following aerosol deposition to the water surface, and 2) evaluate the toxicity of CEES to two species of freshwater algae.

3.6.1 Chemical Fate and Stability in Aquatic Systems

In the preliminary test of aqueous stability, CEES degraded to HEES within seconds; HEES was stable in water up to at least 16 h. Applied as an aerosol to the water surface, CEES rapidly hydrolyzed to HEES and reached an equilibrium between the surface microlayer and water column. Equilibrium was reached within 1 min and did not change significantly thereafter during the subsequent 96-h monitoring period. Concentrations were 3.2 ± 0.6 (s.d.) mg/L and 3.23 ± 4.15 µg/filter for the water and microlayer, respectively. One filter represents 1.91% of the water surface of the tank; therefore, the total surface microlayer of the tank contained approximately 0.169 mg of HEES. The water column (5 L) contained only 16 mg of HEES. The total measured HEES (16.2 mg) was only 1.5% of the applied dose (1075 mg) of CEES. A loss of 98.5% could be due to a combination of adherence to the cover and walls above the water surface, retention in the air brush, and volatilization.

Analysis using the Student t-test on the paired samples (tanks A and B) indicated that concentrations of HEES in the microlayer or water did not differ significantly due to the presence of wind on the water surface.

3.6.2 Effects on Phytoplankton

Generally, growth and final yield of algal cultures exposed to concentrations of CEES greater than 100 mg/L differed significantly from those of control cultures addition (Tables 3.13 and 3.14, and Figure 3.14). Slight stimulation in algal growth occurred at the lower doses of CEES. This is attributed to a commonly recognized compensatory reaction (hormesis) of algae and other organisms exposed to low levels of environmental stress. Linear regression of growth rate versus CEES concentration yields the following relationships:

1) For Chlorella $Y = 270.4 - 1.45\ X$, $r^2 = 0.896$
2) For Selenastrum $Y = 168.5 - 0.78\ X$, $r^2 = 0.861$

where Y = growth rate as percent of control,
X = concentration of CEES (mg/L)

Thus, a concentration of 152 mg/L of CEES results in approximately a 50% reduction in the growth rate of either species.

A surface dose equivalent to 42 g/m^2 of CEES was applied. Recovery data suggested that only 1.5% of this amount deposited on the water surface and remained as HEES. Thus, an actual deposition of 630 mg/m^2 would be expected

TABLE 3.13. Effect of CEES/HEES on Growth Rates and Final Yield of Chlorella

Conc. (mg/L)	Optical Density (replicates) A	B	C	D	E	Percent Mean	Control	T-Test
Growth Rate								
200	0.010	0.010	0.003	0.009	0.009	0.008	3.48	++
180	0.011	0.010	0.004	0.002	0.012	0.008	3.23	++
160	0.049	0.043	0.017	0.033	0.032	0.035	14.42	++
140	0.158	0.162	0.098	0.129	0.089	0.127	52.69	++
120	0.297	0.294	0.275	0.283	0.225	0.275	113.84	-
100	0.311	0.347	0.278	0.325	0.333	0.319	132.0	*
0	0.261	0.258	0.215	0.226	0.247	0.241	100.00	
Final Yield								
200	0.002	0.006	0.008	0.006	0.004	0.005	1.5	++
180	0.004	0.005	0.008	0.007	0.009	0.007	1.93	++
160	0.215	0.211	0.155	0.195	0.167	0.189	55.1	++
140	0.391	0.364	0.348	0.343	0.273	0.344	100.53	-
120	0.410	0.405	0.419	0.407	0.376	0.403	117.95	*
100	0.379	0.413	0.352	0.481	0.406	0.410	119.88	*
0	0.374	0.343	0.315	0.333	0.345	0.342	100.00	

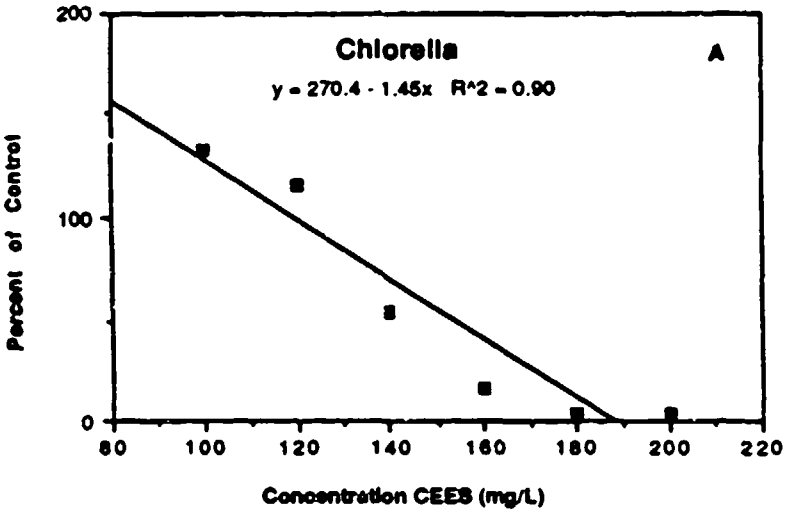
++ = $P \leq 0.01$
+ = $P \leq 0.05$
- = Not significantly different from controls.
* = Significant stimulation of algal growth.

to result in our observed HEES water concentration of 3.2 mg/L. According to Equations 1 and 2 above, significant toxicity (10% reduction in algal growth) occurred at about 100 to 124 mg/L.

TABLE 3.14. Effect of CEES/HEES on Growth Rates and Final Yield of Selenastrum

Conc. (mg/L)	Optical Density (replicates) A	B	C	D	E	Percent Mean	Control	T-Test
Growth Rate								
200	0.018	0.020	0.018	0.020	0.020	0.019	6.34	++
180	0.070	0.045	0.090	0.040	0.042	0.057	18.94	++
160	0.190	0.200	0.200	0.192	0.190	0.194	64.16	++
140	0.212	0.190	0.210	0.201	0.215	0.206	67.8	++
120	0.238	0.200	0.220	0.210	0.220	0.218	71.82	++
100	0.265	0.248	0.260	0.251	0.228	0.250	82.64	++
0	0.340	0.280	0.300	0.290	0.305	0.303	100.00	
Final Yield								
200	0.015	0.020	0.020	0.020	0.024	0.020	3.87	++
180	0.095	0.048	0.520	0.045	0.051	0.152	29.25	+
160	0.570	0.550	0.580	0.575	0.550	0.565	108.86	*
140	0.585	0.530	0.580	0.580	0.580	0.571	110.02	*
120	0.590	0.600	0.580	0.600	0.580	0.590	113.6	*
100	0.625	0.620	0.605	0.630	0.620	0.620	119.46	*
0	0.525	0.530	0.520	0.500	0.520	0.519	100.00	

++ = P ≤0.01
+ = P ≤0.05
- = Not significantly different from controls.
* = Significant stimulation of algal growth.



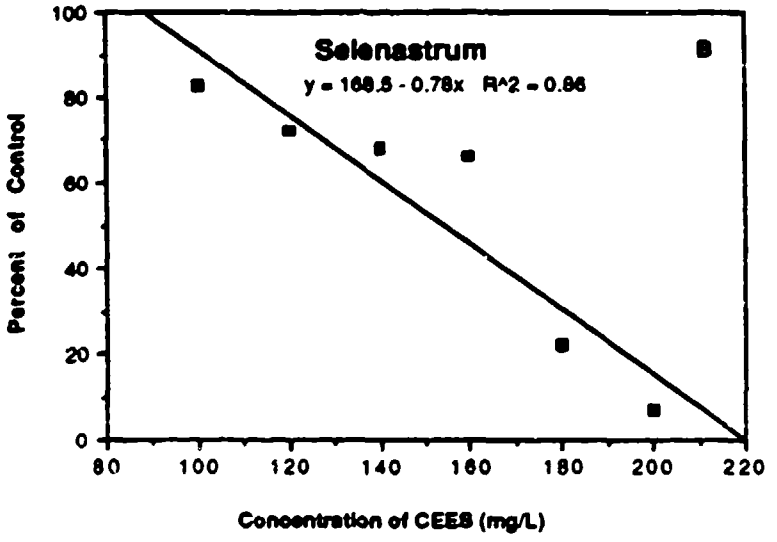


FIGURE 3.14. Effects of CEES/HEES on Growth of (A) Chlorella and (B) Selenastrum

4. CONCLUSIONS

Results for both aerosolized and surface-deposited CEES indicate that its vapor pressure is high enough to result in mixed gas and liquid phases. These affect the overall rate of deposition to surfaces. In addition, the volatility of CEES appears to result in substantial loss of the chemical from foliar surfaces following aerosol application.

The half-lives of CEES and HEES were found to be 2 to 5 and 4 to 8 h, respectively, following deposition to foliar surfaces. The half-life of CEES in soils was found to be 0.9 to 2.4 h. No measurable decline in concentration of HEES was observed over the 96-h treatment period. No VES was found associated with soil or foliar extracts. The half-life of CEES/HEES is substantially less than for DFP and particularly DIMP, which were reported to be approximately 2 and 25 days, respectively (Van Voris et al. 1987).

The phytotoxic effect of CEES appears to have a varied phytotoxic effect among different plant species. Under the protocol followed in these experiments, the pine and the sagebrush were apparently the most sensitive and grass the most tolerant. The simulant appeared to have a contact toxicity and did not seem to affect the onset or rate of new growth unless the initial damage was too severe. Gross damage was comparable to that previously reported for DFP and DIMP (Van Voris et al. 1987). Metabolically, the material exhibited the most dramatic effects on the photosynthetic capability of the plant, although slightly elevated respiration rates were also evident. Within the photosynthetic apparatus in the chloroplasts, those components of the electron transport chain closest to the outside of the thylakoid membrane (PS I and associated carriers) were the first to be affected, producing an uncoupling or rapid elevation in the rate of water splitting and oxygen evolution. This behavior is similar to that noted for DFP (Van Voris et al. 1987). Loss of these components would definitely cause other losses in metabolic capacity leading to the eventual death of the organism if the dose were severe and prolonged.

Results from *in vitro* testing of CEES indicated that concentrations below 10 µg/g dry soil generally did not immediately impact microbial activity in the soil. The one exception was in Palouse soil, dehydrogenase activity showed an immediate response. In general, the extent of enzyme inhibition increased with incubation time with no recovery observed. Palouse soil, with a higher content of organic matter (2.0% organic carbon) than Burbank soil (0.5% organic carbon), seemed more susceptible to enzyme inhibition by CEES. This observation is in contrast with DFP and DIMP studies, in which phosphatase and dehydrogenase activities in Burbank soil were more severely affected than in Palouse soil. Although *in vitro* testing usually gives a good indication of dose response and may be representative of a spill or waste disposal situation, it is not warranted

to be a true representation of actual aerosol deposition. Factors such as mass loading, wind speed, and relative humidity can vary so that when used as a simulant for environmental testing, the influence of CEES on soil microbial and biochemical activity may be different following a depositional event.

Applied as an aerosol to the water surface, CEES rapidly volatilized, though sufficient CEES hydrolyzed that the solubility product of HEES was apparently reached. Toxicity tests using Chlorella and Selenastrum species indicated that a dose of 100 to 124 mg/L results in a 10% reduction in freshwater algal growth. The reported effects of DFP and DIMP on growth of these algae were much more pronounced (Van Voris et al. 1987).

REFERENCES

Arnon, D.I. 1949. "Copper Enzymes in Isolated Chloroplasts. Polyphenyloxidase in Beta vulgaris." Plant Physiol. 24:1-15.

Bennett, Capt. S. 1984. Personal communication. U.S. Army Chemical Research, Development and Engineering Center, Aberdeen Proving Ground, Maryland.

Bossle, P.C., J.J. Martin, E.W. Sarver, and H.Z. Sommer. 1983. High Performance Liquid Chromatography Analysis of 2-Chloroethyl Ethylsulfide and its Decomposition By-Products by Derivatization. Technical Report ARCSL-TR-83027, U.S. Army Armament, R & D Command, Aberdeen Proving Grounds, Maryland.

Bossle, P.C. et al. 1984. "HPLC Analysis of CEES and its Decomposition Byproducts by Derivatization." J.Chromotography. 283: 412-416.

Boyer, J.S., and B.L. Bowen. 1970. "Inhibition of Oxygen Evolution in Chloroplasts Isolated from Leaves with Low Water Potential." Plant Physiol. 45:612-615.

Daubenmire, R. 1959. "A Canopy Cover Method of Vegetation Analysis." N.W. Sciences. 33:43-64.

Keck, R.W., and J.S. Boyer. 1974. "Chloroplast Response to Low Leaf Water Potentials. III. Differing Inhibition of Electron Transport and Photophosphorylation." Plant Physiol. 53:474-479.

Klein, D.A. et al. 1979. "Role of Soil Microorganisms as Indicators and Possible Controlling Factors in Plant Succession Processes on Retorted Shale and Disturbed Soils." In Rehabilitation Potential and Practice of Colorado Oil Shale Lands, ed. C. W. Cook. Colorado State University, Fort Collins, Colorado.

Ramirez-Martinez, J.R. 1968. "Organic Phosphorus Mineralization and Phosphatase Activity in Soils." Folia Microbiol. 13:161.

Reinbold, C.A., Wentsel, R.S., Herricks, E.E., Kerster, H.W., and Shaeffer, D.J. 1986. "Environmental Hazard Ranking of Chemical Agent Simulants." In U.S. Army Chemical Research, Development and Engineering Center Annual Scientific Meeting. U.S. Army Chemical Research, Development and Engineering Center, Aberdeen Proving Ground, Maryland.

Sadowski, L., A. Tucci, and D. Wilson. 1983. Analytical Methods Development for the Separation/Concentration of the Simulant Chloroethyl Ethyl Sulfide from Diethylphthalate. DRXTH-TE-CR-83187, Defense Technical Information Center, U.S. Army Toxic and Hazardous Materials Agency. Aberdeen Proving Grounds, Maryland.

Skujins, J. 1976. "Extracellular Enzymes in Soil." CRC Crit. Rev. Microbiol. 4:383-421.

Tabatabai, M. A. 1982. "Soil Enzymes." In Methods of Soil Analysis, Part 2 (2nd Edition), ed. A. L. Page. Am. Soc. Agronomy, Madison, Wisconsin.

Tabatabai, M. A. and J. M. Bremner. 1969. "Use of p-nitrophenylphosphate for Assay of Soil Phosphatase Activity." Soil Biol. Biochem. 1:301-307.

Van Voris, P., D.A. Cataldo, M.W. Ligotke, J.K. Fredrickson, S. W. Li, E.A. Crecelius, J.T. Hardy, R.J. Fellows, and R.S. Wentsel. 1987. Acute Environmental Toxicity and Persistence of Selected Chemical Agent Simulants: DFP and DIMP. CRDEC-CR-87071, Chemical Research, Development and Engineering Center, Aberdeen Proving Grounds, Maryland.

Walker, D.A. 1980. "Preparation of Higher Plant Chloroplasts." In Methods in Enzymology Vol. 69, ed. A. San Pietro, Academic Press, New York. pp. 94-104.

APPENDIX A

QUALITY ASSURANCE AND QUALITY CONTROL

At Pacific Northwest Laboratory (PNL), quality control (QC) is "the system of activities to provide a quality product," and quality assurance (QA) is "the system of activities to provide assurance that the quality control system is performing adequately." The prime responsibility for QA/QC is placed on the Program Manager, Principal Investigator, and the Task Leaders; however, the QA audit function is maintained outside of the project and is not directly supported by the project. This audit function is performed by Rob Cuello, the Earth and Environmental Science Center's Senior Quality Assurance Officer and is outside the control of the Program Manager or the Principal Investigator.

QUALITY ASSURANCE

The objective of the QA program is to ensure that the production of data are precise and accurate (within the stated acceptance criteria), representative, comparable, traceable, and defensible. To ensure these conditions are met, PNL's quality assurance program is envisioned to consist of the following elements:

- Procedure review and approval [of Standard Operating Procedures (SOP's)]
- Personnel training
- Pretest preparation
- Quality Control of sampling function and analysis function
- QA audits
- Feedback and corrective action (if necessary).

Quality Assurance Training

The activities of the QA training program include: introducing personnel to the QA program, identifying training elements, preparing and training supervisory and test personnel, implementing QA audits, and conducting refresher QC training or on-the-job training as needed.

The project team will review the procedures that govern the collection and interpretation of field and/or laboratory data and will familiarize themselves with what audit procedures are to be used. Supervisory personnel will require some training to become knowledgeable of the QA procedures to be employed.

Procedure Review and Approval (SOP)

Quality control guidelines for a particular program are developed by the

Task Leaders and approved by the Principal Investigator for all standard operating procedures (sampling, analysis and reporting), instruction, specifications, equipment, and so forth. All SOP's for sampling and analysis are described in writing, and each procedure is reviewed to determine what quality control steps will be incorporated. Each team member will use the procedures outlined in the SOP's to prevent contamination, to provide the proper size sample, to assure proper taxonomic identification, to provide proper kind and number of blanks, to maintain standardization of measuring equipment, and to guarantee the keeping of useable records.

Quality assurance also ensures that the documentation system provides for an maintains a current configuration (latest revision) of procedures being used on the program. Whenever it is evident that data being obtained are not sufficiently accurate or appropriate for the intent of the program, the sampling and/or analytical SOP must be modified after the review and approval by the Project Manager. The modifications are then incorporated into the revised SOPs.

Laboratory Record Books

Work performed on any given project is documented in laboratory record books issued to the project personnel. Permanent records on each book are maintained by PNL and include the project number under which the book was issued, the Record Book number, and the name of the staff member to whom the book was issued. A signature sheet for each record book is kept on file with PNL, and an assignment form inside the front cover of the book is used to indicate the project work number and the individual to whom the book was assigned. Staff members assume full responsibility for the use and security of the books while the books are in their possession. At the end of the project, the books are returned to PNL for its archives.

Sample Identification and Traceability

A system of accountability is used to control the number and variety of samples and the quality of data generated on a research project. The Principal Investigator helps design the initial system and, since he or she works most closely with the experiments, helps monitor the system's effectiveness. Quality assurance at this stage of the project is used to assure that the samples are complete and appropriate. Therefore, the QA officer has the following responsibilities:

- Participate in the development of a system for keeping records of samples and for introducing proper QA steps to assure dependability of the system.

- Review sample log system and implementation forms. (The forms provide information on samples collected and indicate the work to be done on the samples.)

- Monitor the sample identification system to ensure proper labeling of samples, proper dispersements of samples for analysis, and proper quality control of spikes, blanks, and duplicates.

The Principal Investigator and the Task Leaders are responsible for seeing that the documentation is done either by themselves or by the team members. A team member is usually assigned the responsibility of handling the samples and storing, retrieving, dispersing, and maintaining records of those samples.

QUALITY CONTROL

The key quality control operations that may be emphasized in a particular program are procurement QA, standardization/calibration, sampling, and analysis.

Procedure Quality Control

Task Leaders are responsible for ensuring that all procured materials (e.g., samples, collection containers) conform to appropriate specifications. They also are responsible for ensuring that reagents and chemicals with limited shelf life are identified and used within the specified expiration date.

Sampling Quality Control

A sampling information document developed by the Task Leader is used to detail the kind of samples to be taken, the locations where samples are taken, the time and duration of sampling, the size of samples to be taken, and other pertinent information on the conditions that are useful to the sampling team. From this information, the sampling team will select the labor hours and apparatus necessary to carry out the sampling task and will follow the appropriate SOP.

The sampling team then develops a QA plan that includes:

- sampling information forms
- lists of apparatus, reagents, supplies
- pre-sampling calibrations
- on-site checks of apparatus
- post-sampling calibrations.

Analytical Quality Control

Analytical quality is monitored through function checks and control checks. Function checks are performed by the analyst to verify the stability and validity of the sample and the performance of the analytical equipment. Sample validity is assessed in terms of spoilage, container integrity, amount of specimen, sample identification, sample blanks, and other general appearance such as condition of filters or uniformity of sample collections across a filter.

The analytical equipment is checked in terms of calibration and performance of calibrating standards; the latter is part of the permanent record of the analysis. It is recommended that calibration standards that span the working range in factors of two should be run through the entire analysis system at least four times. This develops information on precision and detection limits where appropriate.

Control checks are made by analyzing samples provided by the Task Leader. These samples include blanks, duplicates, spikes, and, if available, standard reference materials in quantities that depend on the total number of samples assigned and on the level of accuracy needed in the analysis. These control samples are introduced into the system in such a manner that the analyst will not give them particular attention.

As a rule, large batches of samples (25 samples or more) should have a control (spike, blank, or replicate) sample in every five samples. A small batch of samples (up to five samples) may have more control samples than real samples.

For large and continuous amounts of samples, control charts of performance of duplicates and spikes must be maintained. This allows the analyst and Task Leader to know when the system is out of control, which part of the system is the probably cause, and when and what corrective action is to be taken.

APPENDIX B

EXPERIMENTAL PROTOCOLS

Study Component: CHEMICAL ANALYSIS of 2-CHLOROETHYL ETHYL SULFIDE AND ITS MAJOR HYDROLYSIS PRODUCTS HEES AND VES

PURPOSE

A method was needed and developed to permit rapid and consistent analysis of 2-chloroethyl ethyl sulfide (CEES), HEES (ethyl 2-hydroxethyl sulfide), and VES (vinyl ethylsulfide) in a variety of matrices. The method employed involved use of capillary gas chromatographic mass spectrometry (GC/MS), and circumvented analytical problems inherent in the derivativization and HPLC method of Bossle et al. (1983).

ANALYTICAL METHOD

The GC/MS method employed a Hewlett-Packard 5880 capillary gas chromatograph coupled with a Hewlett-Packard 5970a mass selective detector. The gas chromatograph was operated in the splitless injection mode with a loading time of 0.6 min. The column used was a 30-m fused silica capillary column with a polyethylene glycol liquid phase, cross linked and bonded with wax to the fused silica surface. The chromatograph oven was temperature-programmed from 25°C to 180°C at 8°C/min, with a 4-min hold at the initial temperature. At 180°C the oven temperature was programmed at 20°C/min to a final temperature of 250°C. The injection port and transfer line to the mass spectrometer were set at 250°C. The quadruple mass spectrometer was operated in the selective ion monitoring mode using a standard PFTBA tune.

METHOD CALIBRATION

The parent compound, CEES, and the two decomposition products, HEES and VES were quantified with external standards. Three mass ions were selected for each compound. The criteria for selection was that they were major ions of significant abundance that were free of interferences. For CEES, m/z 75, 124, and 126 were monitored with a dwell time of 50 milliseconds for each ion. For HEES, m/z 61, 75, and 106 were used, and for VES the selected ions were m/z 60, 73 and 88. A 6-point calibration curve was constructed for each compound, with a dynamic range covering three orders of magnitude. Each compound was run in triplicate during calibration, and the best-fit regression line was used to relate the integrated peak area to the mass of compound injected into the mass spectrometer. The detection limit for the three compounds in soil was approximately 10 ng/cm^2, and 1 ng/cm^2 on plant tissues. The difference in order of magnitude in detection limit was a function of the difference in

sample size; in a typical sample, about 10 times more plant surface than soil surface was sampled.

SAMPLE PREPARATION

Hexane extracts of the tissues, soils, deposition coupons, and air samples were transferred to autosample vials and fitted with Teflon-lined crimp-top septa seals. These extracts were analyzed for CEES and its decomposition products without further manipulation.

Study Component: QUANTIFICATION OF PLANT METABOLIC EFFECTS OF CHEMICAL SIMULANTS

PURPOSE

The phytotoxicity of CEES on whole plants was investigated using *in vitro* systems. These included: 1) the effects of the simulant on photosynthesis (oxygen evolution) and dark respiration (oxygen uptake) in intact leaf segments, and 2) the effects of the simulant on specific photochemical reactions and electron transport chains in isolated chloroplasts.

ANALYTICAL METHODS

Whole Leaf Measurements

Leaf samples from the different species exposed at high and low concentrations of the chemical were taken prior to, immediately following, and at several intervals after exposure for analysis of oxygen evolution and uptake. Leaves were excised from the plants, placed in moistened paper towels, and maintained at 4°C until assayed. They were then wet with distilled water and sliced with a razor blade into pieces <5 mm in length or diameter. The pieces were transferred to an assay medium consisting of 2 mM $CaCl_2$, 10 mM sodium bicarbonate, and 20 mM HEPES pH 7.6. Paired tissue samples were taken from this solution and placed directly into paired, water-jacketed (3.9 ml of control media at 20°C) cuvettes. The suspension was continually stirred with magnetic stirrers. The cuvettes were then covered with aluminum foil for dark respiration for approximately 25 min, until a steady-state rate was obtained. They were then illuminated with saturating light (>1200 μEinsteins m^{-2} s^{-1}) at 600 nm for an additional 20 min to obtain a steady-state rate of photosynthesis. After illumination, the tissues were removed from the cuvettes, and blotted and dried overnight in a 75°C oven so the dry weight could be obtained. Assays were run in triplicate and the data expressed as μMol O_2 h^{-1}g dry wt^{-1}.

Isolated Chloroplast Measurements

Chloroplasts were isolated from commercially obtained spinach (*Spinacea oleracea*) leaves according to the methods of Walker (1980). Approximately 80 g of leaves with the mid-ribs removed were washed with distilled water and chilled prior to grinding. The leaves were then ground for 10 s with a sorvall tissue homogenizer in 50 ml of grinding medium, consisting of: 0.33 M sorbitol; 10 mM $Na_4P_2O_7$; 5 mM $MgCl_2$; and 2mM sodium ascorbate, pH 6.5 which had been chilled to a slush-like consistency to maintain the grinding temperature around 4°C. The ground material was then filtered through 8 layers of cheesecloth and the filtrate immediately centrifuged at 1500 x g for 90 s. The supernate was then decanted and the surface of the pellet washed with 1 ml of resuspension mix which was then discarded. The pellet was resuspended in a mixture consisting of 0.3 M sorbitol; 2 mM Na_2EDTA;1 mM $MgCl_2$; 1 mM $MnCl_2$; 50 mM HEPES; 10 mM $NaHCO_3$; 5 mM PP_i; 0.5 mM P_i, pH 7.6. Chlorophyll content was determined according to the method of Arnon (1949): 50 µl of the chloroplast suspension was added to 20 ml of 80% (v/v) acetone and filtered (through No.1 Whatman paper), and the absorbance read at 652 nm. Nine divided by the absorbance gives the volume of the original suspension containing 100 µg of chlorophyll. All procedures were carried out under low light and at 4°C.

Photochemical Assays

Assays were conducted on PS II, PS I, and whole-chain electron transport, measuring oxygen evolution and uptake with a Clark-type electrode (YSI Instruments) in a 1.8-ml volume, water-jacketed cuvette (Gilson Medical Electronics) maintained at 20°C. Stock solution of the CEES was prepared so that addition of 100 µl would equal a final concentration within the cuvette of 1 or 10 ppm. All assays were conducted in paired cuvettes at the same time, with one cuvette serving as a control and the other containing the simulant. The CEES was either added directly to the cuvette prior to illumination (~1 min) or to a chloroplast suspension in a test tube for 1 h prior to transfer to the cuvette for assay. Control chloroplasts were treated in the same manner. Assays were run in triplicate, and all data are expressed in either µMol O_2 $h^{-1}mg^{-1}$chl or as % control of the paired assay. The analyses of the three components of the chloroplast electron transport system were performed according to the following methods:

PS II Measurements

Assays were conducted according to the methods of Boyer and Bowen (1970). The assay medium (1.8 ml) consisted of 0.33 M sorbitol; 2 mM Na_2EDTA; 1 mM $MgCl_2$; 1 mM $MnCl_2$; and 50 mM HEPES, pH 7.6. Sodium

2,6-dichloroindophenol (DCIP), 0.88 mM, was added just prior to the addition of chloroplasts (100 µg). The suspension was then illuminated from the side with saturating light (>1200 µEinsteins $m^{-2}sec^{-1}$) at 600 nm, and the rate of oxygen evolution determined from the initial slope of the electrode output as a function of time.

PS I Measurements

Assays were conducted according to the methods of Keck and Boyer (1974). The assay medium consisted of 1 mM ADP, 1 mM K_2HPO_4, 0.1 M KCl, 5 mM $MgCl_2$, 0.1 mM DCMU, 80 µM DCIP, 1 mM sodium ascorbate, 0.5 mM methyl viologen (MV), 0.5 mM sodium azide (prepared daily), and 100 µg chlorophyll. Assays were illuminated and measured as above.

Whole-Chain (Water to MV) Measurements

Assay conditions were identical to those described for PS I measurements, Keck and Boyer (1974), except that DCMU, DCIP, and sodium ascorbate were deleted from the medium.

Study Component: INHIBITION OF SOIL ENZYMATIC PROCESSES BY CHEMICAL SIMULANTS

PURPOSE

The effects of CEES on soil microbial and biochemical activities were evaluated *in vitro* by measuring the activity of two soil enzymes, dehydrogenase and phosphatase.

DOSING AND INCUBATION

Stock solutions of CEES (Aldrich Cat. No. 24264-0, Lot No. KM00903JM) were prepared in distilled water and added to samples of Palouse and Burbank silt loam soils (to final concentrations ranging from 0 to 250 µg/g dry soil) and incubated at 22°C in the dark. All dehydrogenase and phosphatase activities were measured in duplicate and mean values were compared with those of the control soil (not CEES-treated) and expressed as percent of those of the control.

EXPERIMENTAL METHODS

Dehydrogenase Activity

Soil samples were assayed for dehydrogenase activity as described by Tabatabai (1982) immediately following incubation and after 1 week and 4 weeks. Soils amended with CEES (1.5 g dry weight basis) were first mixed with 0.015 g of $CaCO_3$; 0.3 ml of 1% glucose and 0.25 ml of 3% 2,3,5-triphenyltetrazolium chloride (TTC) and incubated for 24 h at 22°C. Ten ml of methanol was then added to the soil and mixed thoroughly. The mixture was centrifuged and the absorbance of the supernatant at 485 nm was measured using a Beckman DU-50 spectrometer. Soil dehydrogenase activity, expressed as mg of TTC-formazan produced per g of soil/24 h, was quantified by comparing absorbance values to a standard curve prepared with reagent-grade TTC-formazan and methanol.

Phosphatase Activity

Soil phosphatase activity was measured on the CEES-amended soil using the procedure described by Tabatabai and Bremner (1969) as modified by Klein et al. (1979). One g of soil (dry weight) was placed in 15-ml centrifuge tubes with 4 ml of modified universal buffer (MUB), which consists of tris(hydroxymethyl) amino methane, 3.025 g; maleic acid, 2.9 g; citric acid, 3.5 g; boric acid, 1.57 g; 1 N NaOH, 122 ml yielding final volume of pH 8.65. One ml of para-nitrophenol phosphate (0.025 N prepared with MUB) was added to each tube. The tubes were stoppered, vortexed and incubated for 1 h at 37°C. One ml of 0.5 N $CaCl_2$ and 4 ml of 0.5 N NaOH were then added to stop the reaction. The mixtures were centrifuged at 12,000 g for 10 min, and supernatant absorbance was measured at 400 nm with a spectrophotometer. Phosphatase activity was determined by comparing these values to a standard curve constructed with reagent-grade para-nitrophenol and expressed as µg of para-nitrophenol released per g of soil/hour.